

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
3 October 2002 (03.10.2002)

PCT

(10) International Publication Number
WO 02/077263 A2(51) International Patent Classification⁷: **C12Q**

(21) International Application Number: PCT/US02/09039

(22) International Filing Date: 20 March 2002 (20.03.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/278,166 22 March 2001 (22.03.2001) US(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US 60/278,166 (CIP)
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(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KL, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

Published:

- without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **NUCLEIC ACID MOLECULES ENCODING SERINE PROTEASE CVSP14, THE ENCODED POLYPEPTIDES AND METHODS BASED THEREON**

(57) Abstract: Provided herein are polypeptides designated CVSP14 polypeptides that exhibit protease activity as a single chain or as an activated two chain form. Methods using the polypeptides to identify compounds that modulate the protease activity thereof are provided. The polypeptides also serve as tumor markers.



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**NUCLEIC ACID MOLECULES ENCODING SERINE PROTEASE CVSP14, THE
ENCODED POLYPEPTIDES AND METHODS BASED THEREON**

RELATED APPLICATIONS

Benefit of priority is claimed to U.S. provisional application Serial No.

- 5 60/278,166, filed March 22, 2001, to Edwin L. Madison and Jiunn-Chern Yeh
entitled "NUCLEIC ACID MOLECULES ENCODING A TRANSMEMBRANE SERINE
PROTEASE 14, THE ENCODED PROTEINS AND METHODS BASED THEREON."

Where permitted, the subject matter of U.S. provisional application is
incorporated by reference in its entirety.

10 **FIELD OF THE INVENTION**

Nucleic acid molecules that encode proteases and portions thereof,
particularly protease domains are provided. Also provided are prognostic,
diagnostic and therapeutic methods using the proteases and domains thereof and
the encoding nucleic acid molecules.

15 **BACKGROUND OF THE INVENTION AND OBJECTS THEREOF**

- Cancer is a leading cause of death in the United States, developing in one
in three Americans; one of every four Americans dies of cancer. Cancer is
characterized by an increase in the number of abnormal neoplastic cells, which
proliferate to form a tumor mass, the invasion of adjacent tissues by these
20 neoplastic tumor cells, and the generation of malignant cells that metastasize via
the blood or lymphatic system to regional lymph nodes and to distant sites.

- Among the hallmarks of cancer is a breakdown in the communication
among tumor cells and their environment. Normal cells do not divide in the
absence of stimulatory signals, and cease dividing in the presence of inhibitory
25 signals. Growth-stimulatory and growth-inhibitory signals are routinely
exchanged between cells within a tissue. In a cancerous, or neoplastic, state, a
cell acquires the ability to "override" these signals and to proliferate under
conditions in which normal cells do not grow.

- In order to proliferate tumor cells acquire a number of distinct aberrant
30 traits reflecting genetic alterations. The genomes of certain well-studied tumors
carry several different independently altered genes, including activated
oncogenes and inactivated tumor suppressor genes. Each of these genetic

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changes appears to be responsible for imparting some of the traits that, in the aggregate, represent the full neoplastic phenotype.

A variety of biochemical factors have been associated with different phases of metastasis. Cell surface receptors for collagen, glycoproteins such as laminin, and proteoglycans, facilitate tumor cell attachment, an important step in invasion and metastases. Attachment triggers the release of degradative enzymes which facilitate the penetration of tumor cells through tissue barriers. Once the tumor cells have entered the target tissue, specific growth factors are required for further proliferation. Tumor invasion and progression involves a complex series of events, in which tumor cells detach from the primary tumor, break down the normal tissue surrounding it, and migrate into a blood or lymphatic vessel to be carried to a distant site. The breaking down of normal tissue barriers is accomplished by the elaboration of specific enzymes that degrade the proteins of the extracellular matrix that make up basement membranes and stromal components of tissues.

A class of extracellular matrix degrading enzymes have been implicated in tumor invasion. Among these are the matrix metalloproteinases (MMP). For example, the production of the matrix metalloproteinase stromelysin is associated with malignant tumors with metastatic potential (see, *e.g.*, McDonnell *et al.* (1990) *Smnrs. in Cancer Biology* 1:107-115; McDonnell *et al.* (1990) *Cancer and Metastasis Reviews* 9:309-319).

The capacity of cancer cells to metastasize and invade tissue is facilitated by degradation of the basement membrane. Several proteinase enzymes, including the MMPs, have been reported to facilitate the process of invasion of tumor cells. MMPs are reported to enhance degradation of the basement membrane, which thereby permits tumorous cells to invade tissues. For example, two major metalloproteinases having molecular weights of about 70 kDa and 92 kDa appear to enhance ability of tumor cells to metastasize.

Serine Proteases

Serine proteases (SPs) have been implicated in neoplastic disease progression. Most serine proteases, which are either secreted enzymes or are sequestered in cytoplasmic storage organelles, have roles in blood coagulation, wound healing, digestion, immune responses and tumor invasion and metastasis.

5 A class of cell surface proteins designated type II transmembrane serine proteases, which are membrane-anchored proteins with additional extracellular domains, has been identified. As cell surface proteins, they are positioned to play a role in intracellular signal transduction and in mediating cell surface
10 proteolytic events. Other serine proteases can be membrane bound and function in a similar manner. Others are secreted. Many serine proteases exert their activity upon binding to cell surface receptors, and, hence act at cell surfaces. Cell surface proteolysis is a mechanism for the generation of biologically active proteins that mediate a variety of cellular functions.

15 Serine proteases, including secreted and transmembrane serine proteases, have been implicated in processes involved in neoplastic development and progression. While the precise role of these proteases has not been elaborated, serine proteases and inhibitors thereof are involved in the control of many intra- and extracellular physiological processes, including degradative actions in cancer
20 cell invasion, metastatic spread, and neovascularization of tumors, that are involved in tumor progression. It is believed that proteases are involved in the degradation of extracellular matrix (ECM) and contribute to tissue remodeling, and are necessary for cancer invasion and metastasis. The activity and/or expression of some proteases have been shown to correlate with tumor
25 progression and development.

For example, a membrane-type serine protease MTSP1 (also called matriptase; see SEQ ID Nos. 1 and 2 from U.S. Patent No. 5,972,616; and GenBank Accession No. AF118224; (1999) *J. Biol. Chem.* 274:18231-18236; U.S. Patent No. 5,792,616; see, also Takeuchi (1999) *Proc. Natl. Acad. Sci.*
30 *U.S.A.* 96:11054-1161) that is expressed in epithelial cancer and normal tissue (Takeuchi *et al.* (1999) *Proc. Natl. Acad. Sci. USA* 96:11054-61) has been identified. Matriptase was originally identified in human breast cancer cells as a

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major gelatinase (see, U.S. Patent No. 5,482,848), a type of matrix metalloprotease (MMP). It has been proposed that it plays a role in the metastasis of breast cancer. Matriptase also is expressed in a variety of epithelial tissues with high levels of activity and/or expression in the human gastrointestinal tract and the prostate. MTSPs, designated MTSP3, MTSP4, MTSP6 have been described in published International PCT application No. WO 01/57194, based in International PCT application No. PCT/US01/03471.

Prostate-specific antigen (PSA), a kallikrein-like serine protease, degrades extracellular matrix glycoproteins fibronectin and laminin, and, has been postulated to facilitate invasion by prostate cancer cells (Webber *et al.* (1995) *Clin. Cancer Res.* 1:1089-94). Blocking PSA proteolytic activity with PSA-specific monoclonal antibodies results in a dose-dependent decrease *in vitro* in the invasion of the reconstituted basement membrane Matrigel by LNCaP human prostate carcinoma cells which secrete high levels of PSA.

Hepsin, a cell surface serine protease identified in hepatoma cells, is overexpressed in ovarian cancer (Tanimoto *et al.* (1997) *Cancer Res.* 57:2884-7). The hepsin transcript appears to be abundant in carcinoma tissue and is almost never expressed in normal adult tissue, including normal ovary. It has been suggested that hepsin is frequently overexpressed in ovarian tumors and therefore can be a candidate protease in the invasive process and growth capacity of ovarian tumor cells.

A serine protease-like gene, designated normal epithelial cell-specific 1 (NES1) (Liu *et al.*, *Cancer Res.*, 56:3371-9 (1996)) has been identified. Although expression of the NES1 mRNA is observed in all normal and immortalized nontumorigenic epithelial cell lines, the majority of human breast cancer cell lines show a drastic reduction or a complete lack of its expression. The structural similarity of NES1 to polypeptides known to regulate growth factor activity and a negative correlation of NES1 expression with breast oncogenesis suggest a direct or indirect role for this protease-like gene product in the suppression of tumorigenesis.

Hence transmembrane and other serine proteases and other proteases appear to be involved in the etiology and pathogenesis of tumors. There is a

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need to further elucidate their role in these processes and to identify additional transmembrane proteases. Therefore, it is an object herein to provide serine protease proteins and nucleic acids encoding such proteases that are involved in the regulation of or participate in tumorigenesis and/or carcinogenesis. It is also
5 an object herein to provide prognostic, diagnostic, therapeutic screening methods using such proteases and the nucleic acids encoding such proteases.

SUMMARY OF THE INVENTION

Provided herein is a protein designated CVSP14, including the protease domain thereof (see, *e.g.*, SEQ ID Nos. 5, 6, 12 and 13). CVSP14 is a secreted
10 serine protease. CVSP14 is highly expressed in androgen-independent prostate tumors and is expressed in other tumors. Hence, as a protease it can be involved in tumor progression. By virtue of its functional activity it can be a therapeutic or diagnostic target. The expression and/or activation (or reduction in level of expression or activation) of the expressed protein or zymogen form
15 thereof can be used to monitor cancer and cancer therapy. For example, the expression of the this protein can be used to monitor prostate cancer and prostate cancer therapy.

The serine protease family includes members that are activated and/or expressed in tumor cells at different levels from non-tumor cells; and those from
20 cells in which substrates therefor differ in tumor cells from non-tumor cells or otherwise alter the specificity or activity of the serine protease (SP). The serine protease provided herein, designated herein as CVSP14, is a secreted protease. The protease domain and full-length protein, including the zymogen and activated forms, and uses thereof are also provided. Proteins encoded by splice
25 variants are also provided. Nucleic acid molecules encoding the proteins and protease domains are also provided. The protease domain of a CVSP14 is set forth in SEQ ID No. 6; the sequence of a full length protein is set forth in SEQ ID No. 13. The sequences of encoding nucleic acid molecules are set forth in SEQ ID Nos. 5 and 12, respectively.

30 CVSP14 is expressed as a secreted protein and may bind to cell surface receptors and function as a cell-surface bound protease, such as by binding

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thereto or by dimerization or multimerization with a membrane-bound or receptor-bound protein.

Also provided herein are nucleic acid molecules that encode SP proteins and the encoded proteins. In particular, nucleic acid molecules encoding
5 CVSP14 from animals, including splice variants thereof are provided. The encoded proteins are also provided. Also provided are functional domains thereof. For example, the SP protease domains, portions thereof, and muteins thereof are from or based on animal SPs, including, but are not limited to, rodent, such as mouse and rat; fowl, such as chicken; ruminants, such as goats,
10 cows, deer, sheep; ovine, such as pigs; and humans.

The protease domain for use in the methods and assay provided herein does not have to result from activation, which produces a two chain activated product, but rather is a single chain polypeptide where the N-terminus includes the sequence ↓ILGG. Such polypeptides, although not the result of activation
15 and not two-chain forms, exhibit proteolytic (catalytic) activity. These protease domain polypeptides are used in assays to screen for agents that modulate the activity of the CVSP14.

Such assays are also provided herein. In exemplary assays, the effects of test compounds on the ability of the full length or along at least about 70%,
20 80% or 90% of the full length of the single chain, two chain activated form, or a protease domain, which is a single chain or a two chain activated form, of CVSP14 to proteolytically cleave a known substrate, typically a fluorescently, chromogenically or otherwise detectably labeled substrate, are assessed. Agents, generally compounds, particularly small molecules, that modulate the
25 activity of the protein (full length or protease domain either single or two chain forms thereof) are candidate compounds for modulating the activity of the CVSP14. The protease domains and full length proteins also can be used to produce two-chain and single-chain protease-specific antibodies. The protease domains provided herein include, but are not limited to, the single chain region
30 having an N-terminus at the cleavage site for activation of the zymogen, through the C-terminus, or C-terminal truncated portions thereof that exhibit proteolytic activity as a single-chain polypeptide in *in vitro* proteolysis assays, of any family

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member, including CVSP14, such as from a mammal, including human, that, for example, is expressed or activity in tumor cells at different levels from non-tumor cells.

Also provided are muteins of the single chain protease domain of CVSP14 particularly muteins in which the Cys residue (residue no. 26 in SEQ ID No. 6) in the protease domain that is free (*i.e.*, does not form disulfide linkages with any other Cys residue in the protease domain) is substituted with another amino acid substitution, generally with a substitution that does not eliminate the activity of interest, and muteins in which a glycosylation site(s) is eliminated. Muteins in which other substitutions in which catalytic activity is retained are also contemplated (see, *e.g.*, Table 1, for exemplary amino acid substitutions).

Hence, provided herein is a member of the family of serine proteases designated CVSP14, and functional domains, especially protease (or catalytic) domains thereof, muteins and other derivatives and analogs thereof. Also provided herein are nucleic acids encoding the CVSP14.

The nucleic acid and amino acid sequences of CVSP14 are set forth in SEQ ID Nos. 5 and 6. Nucleic acid molecules that encode a single-chain protease domain or catalytically active portion thereof and also those that encode the full-length CVSP14 (SEQ ID Nos. 12 and 13) are provided. Single amino acid changes are contemplated; for example peptides in which there is an Arg in place of a Gly are provided. Nucleic acid molecules that encode a single-chain protease domain or catalytically active portion thereof and also those that encode the full-length CVSP14 are provided. Also provided are nucleic acid molecules that hybridize to such CVSP14 encoding nucleic acid along their full length or along at least about 70%, 80% or 90% of the full length and encode the full length or a truncated portion thereof, such as without the signal sequence or a protease domain or catalytically active portion thereof are provided. Hybridization is typically performed under conditions of at least low, generally at least moderate, and often high stringency.

Additionally provided herein are antibodies that specifically bind to the CVSP14 and inhibit the activity thereof. Included are antibodies that specifically bind to the protein or protease domain, including to the single and/or two chain

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forms thereof. Among the antibodies are two-chain-specific antibodies, and single-chain specific antibodies and neutralizing antibodies. Antibodies that specifically bind to the CVSP14, particularly the single chain protease domain, the zymogen and activated form are also provided herein. Antibodies that
5 specifically bind to the two-chain and/or single-chain form of CVSP14 are provided. The antibodies include those that specifically bind to the two-chain or single-chain form of the protease domain and/or the full-length protein.

Further provided herein are prognostic, diagnostic, therapeutic screening methods using CVSP14 and the nucleic acids encoding CVSP14. Also provided
10 are transgenic non-human animals bearing inactivated genes encoding the CVSP and bearing the genes encoding the CVSP14 under non-native or native promotor control are provided. Such animals are useful in animal models of tumor initiation, growth and/or progression models.

Provided herein are members of a family of serine proteases (SPs) that are
15 expressed in certain tumor or cancer cells such lung, prostate, colon and breast cancers. In particular, it is shown herein, that CVSP14 is expressed in lung carcinoma, leukemia and cervical carcinoma as well as in certain normal cells and tissues (see *e.g.*, EXAMPLES for tissue-specific expression profile). CVSP14 can also be a marker for breast, prostate and colon cancer.

20 SPs are of interest because they appear to be expressed and/or activated at different levels in tumor cells from normal cells, or have functional activity that is different in tumor cells from normal cells, such as by an alteration in a substrate therefor, or a cofactor. CVSP14 is of interest because it is expressed or is active in tumor cells. Hence the CVSP14 provided herein can serve as
25 diagnostic markers for certain tumors. The level of activated CVSP14 can be diagnostic of prostate, uterine, lung or colon cancer or leukemia or other cancer.

Also provided herein are methods of modulating the activity of the CVSP14 and screening for compounds that modulate, including inhibit, antagonize, agonize or otherwise alter the activity of the CVSP14. Of particular
30 interest is the protease domain of CVSP14 that includes the catalytic portion of the protein.

CVSP14 polypeptides, including, but not limited to splice variants thereof, and nucleic acids encoding CVSPs, and domains, derivatives and analogs thereof are provided herein. Single chain protease domains that contain the N-terminii that are generated by activation of the zymogen form of CVSP14 are also
5 provided. The cleavage site for the protease domain is at amino acid 52 (R↓IGGS)(see SEQ ID Nos. 12 and 13).

Also provided are plasmids containing any of the nucleic acid molecules provided herein. Cells containing the plasmids are also provided. Such cells include, but are not limited to, bacterial cells, yeast cells, fungal cells, plant cells,
10 insect cells and animal cells. In addition to cells and plasmids containing nucleic acid encoding the CVSP14 polypeptide, methods of expression of the encoded polypeptide are provided. In order to achieve expression of the protease domain, the nucleic acid encoding the signal sequence is removed. The protein is expressed in the inclusion bodies. The CVSP14 protease domain was then
15 isolated from the inclusion bodies and treated under conditions whereby proper refolding occurred. Hence also provided are methods for producing active CVSP14 protease domain.

Also provided is a method of producing CVSP14 by growing the above-described cells under conditions whereby the CVSP14 is expressed by the cells,
20 and recovering the expressed CVSP14 polypeptide. Methods for isolating nucleic acid encoding other CVSP14s are also provided.

Also provided are cells, generally eukaryotic cells, such as mammalian cells and yeast cells, in which the CVSP14 polypeptide is expressed by the cells. Such cells to which the secreted protein can bind are used in drug screening
25 assays to identify compounds that modulate the activity of the CVSP14 polypeptide. These assays include *in vitro* binding assays, and transcription based assays in which signal transduction mediated directly or indirectly, such as via activation of pro-growth factors, by the CVSP14 or cleavage products thereof is assessed.

30 Further provided herein are prognostic, diagnostic and therapeutic screening methods using the CVSP14 and the nucleic acids encoding CVSP14. In particular, the prognostic, diagnostic and therapeutic screening methods are

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used for preventing, treating, or for finding agents useful in preventing or treating, tumors or cancers such as lung carcinoma, colon adenocarcinoma and ovarian carcinoma.

Also provided are methods for screening for compounds that modulate the activity of CVSP14. The compounds are identified by contacting them with the CVSP14 or protease domain thereof and a substrate for the CVSP14. A change in the amount of substrate cleaved in the presence of the compounds compared to that in the absence of the compound indicates that the compound modulates the activity of the CVSP14. Such compounds are selected for further analyses or for use to modulate the activity of the CVSP14; such as inhibitors or agonists. The compounds also can be identified by contacting the substrates with a cell that binds to a CVSP14 or catalytically active portion thereof.

Also provided herein are modulators of the activity of CVSP14, especially the modulators obtained according to the screening methods provided herein. Such modulators can have use in treating cancerous conditions and other neoplastic conditions.

Pharmaceutical composition containing the protease domain and/or full-length or other domain of a CVSP14 polypeptide are provided herein in a pharmaceutically acceptable carrier or excipient are provided herein.

Also provided are articles of manufacture that contain CVSP14 polypeptide and protease domains of CVSP14 in single chain forms or activated forms. The articles contain a) packaging material; b) the polypeptide (or encoding nucleic acid), particularly the single chain protease domain thereof; and c) a label indicating that the article is for using in assays for identifying modulators of the activities of a CVSP14 polypeptide is provided herein.

Conjugates containing a) a CVSP14 polypeptide or protease domain in single chain form; and b) a targeting agent linked to the CVSP directly or via a linker, wherein the agent facilitates: i) affinity isolation or purification of the conjugate; ii) attachment of the conjugate to a surface; iii) detection of the conjugate; or iv) targeted delivery to a selected tissue or cell, is provided herein. The conjugate can contain a plurality of agents linked thereto. The conjugate can be a chemical conjugate; and it can be a fusion protein.

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In another embodiment, the targeting agent is a protein or peptide fragment. The protein or peptide fragment can include a protein binding sequence, a nucleic acid binding sequence, a lipid binding sequence, a polysaccharide binding sequence, or a metal binding sequence.

5 Methods of diagnosing a disease or disorder characterized by detecting an aberrant level of a CVSP14 in a subject is provided. The method can be practiced by measuring the level of the DNA, RNA, protein or functional activity of the CVSP14. An increase or decrease in the level of the DNA, RNA, protein or functional activity of the CVSP, relative to the level of the DNA, RNA, protein
10 or functional activity found in an analogous sample not having the disease or disorder (or other suitable control) is indicative of the presence of the disease or disorder in the subject or other relative any other suitable control.

Combinations are provided herein. The combination can include: a) an inhibitor of the activity of a CVSP14; and b) an anti-cancer treatment or agent.
15 The CVSP inhibitor and the anti-cancer agent can be formulated in a single pharmaceutical composition or each is formulated in a separate pharmaceutical composition. The CVSP14 inhibitor can be an antibody or a fragment or binding portion thereof made against the CVSP14, such as an antibody that specifically binds to the protease domain, an inhibitor of CVSP14 production, or an inhibitor
20 of CVSP14 membrane-localization or an inhibitor of CVSP14 activation. Other CVSP14 inhibitors include, but are not limited to, an antisense nucleic acid or double-stranded RNA (dsRNA), such as RNAi, encoding the CVSP14 or portions thereof, particularly a portion of the protease domain, a nucleic acid encoding at least a portion of a gene encoding the CVSP14 with a heterologous nucleotide
25 sequence inserted therein such that the heterologous sequence inactivates the biological activity encoded CVSP14 or the gene encoding it. The portion of the gene encoding the CVSP14 typically flanks the heterologous sequence to promote homologous recombination with a genomic gene encoding the CVSP14.

Also, provided are methods for treating or preventing a tumor or cancer in
30 a mammal by administering to a mammal an effective amount of an inhibitor of a CVSP14, whereby the tumor or cancer is treated or prevented. The CVSP14 inhibitor used in the treatment or for prophylaxis is administered with a

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pharmaceutically acceptable carrier or excipient. The mammal treated can be a human. The treatment or prevention method can additionally include administering an anti-cancer treatment or agent simultaneously with or subsequently or before administration of the CVSP14 inhibitor.

- 5 Also provided is a recombinant non-human animal in which an endogenous gene of a CVSP14 has been deleted or inactivated by homologous recombination or other recombination events or insertional mutagenesis of the animal or an ancestor thereof. A recombinant non-human animal is provided herein, where the gene of a CVSP14 is under control of a promoter that is not
10 the native promoter of the gene or that is not the native promoter of the gene in the non-human animal or where the nucleic acid encoding the CVSP14 is heterologous to the non-human animal and the promoter is the native or a non-native promoter or the CVSP14 is on an extrachromosomal element, such as a plasmid or artificial chromosome. Transgenic non-human animals bearing the
15 genes encoding the CVSP14 and bearing inactivated genes encoding CVSP14, particularly under a non-native promoter control or on an exogenous element, such as a plasmid or artificial chromosome, are additionally provided herein.

- Also provided are methods of treatments of tumors by administering a prodrug that is activated by CVSP14 that is expressed or active in tumor cells,
20 particularly those in which its functional activity in tumor cells is greater than in non-tumor cells. The prodrug is administered and, upon administration, active CVSP14 cleaves the prodrug and releases active drug in the vicinity of the tumor cells. The active anti-cancer drug accumulates in the vicinity of the tumor. This is particularly useful in instances in which CVSP14 is expressed or active in
25 greater quantity, higher level or predominantly in tumor cells compared to other cells.

- Also provided are methods of identifying a compound that binds to the single-chain or two-chain form of CVSP14, by contacting a test compound with a both forms; determining to which form the compound binds; and if it binds to a
30 form of CVSP14, further determining whether the compound has at least one of the following properties:

(i) inhibits activation of the single-chain zymogen form of CVSP14;

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(ii) inhibits activity of the two-chain or single-chain form; and

(iii) inhibits dimerization of the protein.

The forms can be full length or truncated forms, including but not limited to, the protease domain resulting from cleavage at the RI activation site or from

5 expression of the protease domain or catalytically active portions thereof.

Also provided are methods of diagnosing the presence of a pre-malignant lesion, a malignancy, or other pathologic condition in a subject, by obtaining a biological sample from the subject; exposing it to a detectable agent that binds to a two-chain or single-chain form of CVSP14, where the pathological condition
10 is characterized by the presence or absence of the two-chain or single-chain form.

Methods of inhibiting tumor invasion or metastasis or treating a malignant or pre-malignant condition by administering an agent that inhibits activation of the zymogen form of CVSP14 or an activity of the activated form are provided.

15 The conditions include, but are not limited to, a condition, such as a tumor, of the breast, cervix, prostate, lung, ovary or colon.

Methods for monitoring tumor progression and/or therapeutic effectiveness are also provided. The levels of activation or expression of CVSP14 or the protease domain thereof are assessed, and the change in the
20 level, reflects tumor progression and/or the effectiveness of therapy. Generally, as the tumor progresses the amount of CVSP14 in a body tissue or fluid sample increases; effective therapy reduces the level.

DETAILED DESCRIPTION OF THE INVENTION

A. DEFINITIONS

25 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the invention(s) belong. All patents, patent applications, published applications and publications, Genbank sequences, websites and other published materials referred to throughout the entire disclosure herein, unless noted
30 otherwise, are incorporated by reference in their entirety. In the event that there are a plurality of definitions for terms herein, those in this section prevail. Where reference is made to a URL or other such identifier or address, it

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understood that such identifiers can change and particular information on the internet can come and go, but equivalent information can be found by searching the internet. Reference thereto evidences the availability and public dissemination of such information.

5 As used herein, the abbreviations for any protective groups, amino acids and other compounds, are, unless indicated otherwise, in accord with their common usage, recognized abbreviations, or the IUPAC-IUB Commission on Biochemical Nomenclature (see, (1972) *Biochem.* 11:942-944).

10 As used herein, serine protease refers to a diverse family of proteases wherein a serine residue is involved in the hydrolysis of proteins or peptides. The serine residue can be part of the catalytic triad mechanism, which includes a serine, a histidine and an aspartic acid in the catalysis, or be part of the hydroxyl/ ϵ -amine or hydroxyl/ α -amine catalytic dyad mechanism, which involves a serine and a lysine in the catalysis. Of particular interest are SPs of
15 mammalian, including human, origin. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, *e.g.*, Watson *et al.* (1987) *Molecular Biology of the Gene*, 4th Edition, The Bejacmin/Cummings Pub. co., p.224).

20 As used herein, "transmembrane serine protease (MTSP)" refers to a family of transmembrane serine proteases that share common structural features as described herein (see, also Hooper *et al.* (2001) *J. Biol. Chem.* 276:857-860). Thus, reference, for example, to "MTSP" encompasses all proteins encoded by the MTSP gene family, including but are not limited to: MTSP3, MTSP4,
25 MTSP6, MTSP7 or an equivalent molecule obtained from any other source or that has been prepared synthetically or that exhibits the same activity. Other MTSPs include, but are not limited to, corin, enterpeptidase, human airway trypsin-like protease (HAT), MTSP1, TMPRSS2, and TMPRSS4. Sequences of encoding nucleic molecules and the encoded amino acid sequences of exemplary
30 MTSPs and/or domains thereof are set forth, for example in U.S. application Serial No. 09/776,191 (SEQ ID Nos. 1-12, 49, 50 and 61-72 therein, published as International PCT application No. WO 01/57194). The term also encompass

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MTSPs with amino acid substitutions that do not substantially alter activity of each member, and also encompasses splice variants thereof. Suitable substitutions, including, although not necessarily, conservative substitutions of amino acids, are known to those of skill in this art and can be made without
5 eliminating the biological activity, such as the catalytic activity, of the resulting molecule.

As used herein, a "protease domain of a CVSP" refers to a domain of CVSP that exhibits proteolytic activity and shares homology and structural features with the chymotrypsin/trypsin family protease domains. Hence it is at
10 least the minimal portion of the domain that exhibits proteolytic activity as assessed by standard *in vitro* assays. Those of skill in this art recognize that such protease domain is the portion of the protease that is structurally equivalent to the trypsin or chymotrypsin fold. Contemplated herein are such protease domains and catalytically active portions thereof. Also provided are truncated
15 forms of the protease domain that include the smallest fragment thereof that acts catalytically as a single chain form.

As used herein, the catalytically active domain of a CVSP refers to the protease domain. Reference to the protease domain of a CVSP includes refers to the single chain form of the protein. If the two-chain form or both is intended, it
20 is so-specified. The zymogen form of each protein is a single chain, which is converted to the active two chain form by activation cleavage.

As used herein a protease domain of a CVSP14, whenever referenced herein, includes at least one or all of or any combination of or a catalytically active portion of:

25 a polypeptide encoded by the sequence of nucleotides set forth in SEQ ID No. 5;

a polypeptide encoded by a sequence of nucleotides that hybridizes under conditions of low, moderate or high stringency to the sequence of nucleotides set forth in SEQ ID No. 5;

30 a polypeptide that comprises the sequence of amino acids set forth in SEQ ID No. 6;

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a polypeptide that comprises a sequence of amino acids having at least about 60%, 70%, 80%, 90% or about 95% sequence identity with the sequence of amino acids set forth in SEQ ID No. 6; and/or

a protease domain of a splice variant of the CVSP14.

5 The CVSP14 can be from any animal, particularly a mammal, and includes but are not limited to, humans, rodents, fowl, ruminants and other animals. The full length zymogen or two-chain activated form is contemplated or any domain thereof, including the protease domain, which can be a two-chain activated form, or a single chain form.

10 By active form is meant a form active *in vivo* and/or *in vitro*. As described herein, the protease domain also can exist as a two-chain form. It is shown herein that, at least *in vitro*, the single chain forms of the SPs and the catalytic domains or proteolytically active portions thereof (typically C-terminal truncations) thereof exhibit protease activity. Hence provided herein are isolated
15 single chain forms of the protease domains of SPs and their use in *in vitro* drug screening assays for identification of agents that modulate the activity thereof.

As used herein, activation cleavage refers to the cleavage of the protease at the N-terminus of the protease domain (in this instance between R₅₅ and I₅₆; with reference to SEQ ID Nos. 12 and 13). By virtue of the Cys-Cys pairing
20 between the a Cys outside the protease domain (in this instance C₃₇) and a Cys in the protease domain (in this instance Cys₁₆₆), upon cleavage the resulting polypeptide has two chains ("A" chain and the "B" chain, which is the protease domain). Cleavage can be effected by another protease or autocatalytically.

As used herein, a two-chain form of the protease domain refers to a two-
25 chain form that is formed from the two-chain form of the protease in which the Cys pairing between, in this instance, Cys₃₇ and Cys₁₆₆, which links the protease domain to the remainder of the polypeptide, the "A" chain. A two chain protease domain form refers to any form in which the "remainder of the polypeptide", *i.e.*, "A" chain, is shortened and includes at least up to Cys₃₇.

30 As used herein a CVSP14, whenever referenced herein, includes at least one or all of or any combination of:

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a polypeptide encoded by the sequence of nucleotides set forth in SEQ ID No. 12;

a polypeptide encoded by a sequence of nucleotides that hybridizes under conditions of low, moderate or high stringency to the sequence of nucleotides set forth in SEQ ID No. 12;

a polypeptide that comprises the sequence of amino acids set forth in SEQ ID No. 13;

a polypeptide that comprises a sequence of amino acids having at least about 60%, 70%, 80%, 90% or about 95% sequence identity with the sequence of amino acids set forth in SEQ ID No. 6 or 13; and/or

a splice variant of the CVSP14.

The CVSP14 polypeptide includes the sequence of amino acids set forth in SEQ ID No. 13. Smaller portions thereof that retain protease activity are contemplated. The protease domain thereof is set forth in SEQ ID No. 6. The protease domains of CVSPs vary in size and constitution, including insertions and deletions in surface loops. They retain conserved structure, including at least one of the active site triad, primary specificity pocket, oxyanion hole and/or other features of serine protease domains of proteases. Thus, for purposes herein, the protease domain is a portion of a CVSP, as defined herein, and is homologous to a domain of other CVSP. As with the larger class of enzymes of the chymotrypsin (S1) fold (see, *e.g.*, Internet accessible MEROPS data base), the CVSPs protease domains share a high degree of amino acid sequence identity. The His, Asp and Ser residues necessary for activity are present in conserved motifs. The activation site, whose cleavage creates the N-terminus of protease domain in the two-chain forms has a conserved motif and readily can be identified.

CVSPs of interest include those that are activated and/or expressed in tumor cells at different levels, typically higher, from non-tumor cells; and those from cells in which substrates therefor differ in tumor cells from non-tumor cells or differ with respect to substrates, co-factors or receptors, or otherwise alter the activity or specificity of the CVSP.

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As used herein, a human protein is one encoded by nucleic acid, such as DNA, present in the genome of a human, including all allelic variants and conservative variations as long as they are not variants found in other mammals.

As used herein, a "nucleic acid encoding a protease domain or
5 catalytically active portion of a SP" shall be construed as referring to a nucleic acid encoding only the recited single chain protease domain or active portion thereof, and not the other contiguous portions of the SP as a continuous sequence.

As used herein, catalytic activity refers to the activity of the SP as a
10 serine protease. Function of the SP refers to its function in tumor biology, including promotion of or involvement in initiation, growth or progression of tumors, and also roles in signal transduction. Catalytic activity refers to the activity of the SP as a protease as assessed in *in vitro* proteolytic assays that detect proteolysis of a selected substrate.

As used herein, a zymogen is an inactive precursor of a proteolytic
15 enzyme. Such precursors are generally larger, although not necessarily larger than the active form. With reference serine proteases zymogens are converted to active enzymes by specific cleavage, including catalytic and autocatalytic cleavage, or binding of an activating co-factor, which generates the mature
20 active enzyme. A zymogen, thus, is an enzymatically inactive protein that is converted to a proteolytic enzyme by the action of an activator.

As used herein, "disease or disorder" refers to a pathological condition in an organism resulting from, *e.g.*, infection or genetic defect, and characterized by identifiable symptoms.

As used herein, neoplasm (neoplasia) refers to abnormal new growth, and
25 thus means the same as *tumor*, which can be benign or malignant. Unlike *hyperplasia*, neoplastic proliferation persists even in the absence of the original stimulus.

As used herein, neoplastic disease refers to any disorder involving cancer,
30 including tumor development, growth, metastasis and progression.

As used herein, cancer refers to a general term for diseases caused by any type of malignant tumor.

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As used herein, malignant, as applies to tumors, refers to primary tumors that have the capacity of *metastasis* with loss of *growth control* and *positional control*.

As used herein, an anti-cancer agent (used interchangeable with "anti-tumor or anti-neoplastic agent") refers to any agents used in the anti-cancer treatment. These include any agents, when used alone or in combination with other compounds, that can alleviate, reduce, ameliorate, prevent, or place or maintain in a state of remission of clinical symptoms or diagnostic markers associated with neoplastic disease, tumor and cancer, and can be used in methods, combinations and compositions provided herein. Non-limiting examples of anti-neoplastic agents include anti-angiogenic agents, alkylating agents, antimetabolite, certain natural products, platinum coordination complexes, anthracenediones, substituted ureas, methylhydrazine derivatives, adrenocortical suppressants, certain hormones, antagonists and anti-cancer polysaccharides.

As used herein, a splice variant refers to a variant produced by differential processing of a primary transcript of genomic nucleic acid, such as DNA, that results in more than one type of mRNA. Splice variants of SPs are provided herein.

As used herein, angiogenesis is intended to broadly encompass the totality of processes directly or indirectly involved in the establishment and maintenance of new vasculature (neovascularization), including, but not limited to, neovascularization associated with tumors.

As used herein, anti-angiogenic treatment or agent refers to any therapeutic regimen and compound, when used alone or in combination with other treatment or compounds, that can alleviate, reduce, ameliorate, prevent, or place or maintain in a state of remission of clinical symptoms or diagnostic markers associated with undesired and/or uncontrolled angiogenesis. Thus, for purposes herein an anti-angiogenic agent refers to an agent that inhibits the establishment or maintenance of vasculature. Such agents include, but are not limited to, anti-tumor agents, and agents for treatments of other disorders

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associated with undesirable angiogenesis, such as diabetic retinopathies, restenosis, hyperproliferative disorders and others.

As used herein, non-anti-angiogenic anti-tumor agents refer to anti-tumor agents that do not act primarily by inhibiting angiogenesis.

- 5 As used herein, pro-angiogenic agents are agents that promote the establishment or maintenance of the vasculature. Such agents include agents for treating cardiovascular disorders, including heart attacks and strokes.

- As used herein, undesired and/or uncontrolled angiogenesis refers to pathological angiogenesis wherein the influence of angiogenesis stimulators
10 outweighs the influence of angiogenesis inhibitors. As used herein, deficient angiogenesis refers to pathological angiogenesis associated with disorders where there is a defect in normal angiogenesis resulting in aberrant angiogenesis or an absence or substantial reduction in angiogenesis.

- As used herein, the protease domain of an SP protein refers to the
15 protease domain of an SP that exhibits proteolytic activity. Hence it is at least the minimal portion of the protein that exhibits proteolytic activity as assessed by standard assays *in vitro*. It refers, herein, to a single chain form and also the two chain activated form (where the two chain form is intended it will be so-noted). Exemplary protease domains include at least a sufficient portion of
20 sequences of amino acids set forth in SEQ ID No. 6 (encoded by nucleotides in SEQ ID No. 5) to exhibit protease activity.

- Also contemplated are nucleic acid molecules that encode a polypeptide that has proteolytic activity in an *in vitro* proteolysis assay and that have at least 60%, 70%, 80%, 90% or about 95% sequence identity with the full length of a
25 protease domain of a CVSP14 polypeptide, or that hybridize along their full length or along at least about 70%, 80% or 90% of the full length to a nucleic acids that encode a protease domain, particularly under conditions of moderate, generally high, stringency.

- For the protease domains, residues at the N-terminus can be critical for
30 activity. It is shown herein that the protease domain of the single chain form of the CVSP14 protease is catalytically active. Hence the protease domain generally requires the N-terminal amino acids thereof for activity; the C-terminus

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portion can be truncated. The amount that can be removed can be determined empirically by testing the polypeptide for protease activity in an *in vitro* assay that assesses catalytic cleavage.

Hence smaller portions of the protease domains, particularly the single
5 chain domains, thereof that retain protease activity are contemplated. Such smaller versions generally are C-terminal truncated versions of the protease domains. The protease domains vary in size and constitution, including insertions and deletions in surface loops. Such domains exhibit conserved structure, including at least one structural feature, such as the active site triad,
10 primary specificity pocket, oxyanion hole and/or other features of serine protease domains of proteases. Thus, for purposes herein, the protease domain is a single chain portion of a CVSP14, as defined herein, but is homologous in its structural features and retention of sequence of similarity or homology the protease domain of chymotrypsin or trypsin. The polypeptide exhibits
15 proteolytic activity as a single chain.

As used herein, by homologous means about greater than 25% nucleic acid sequence identity, such as 25%, 40%, 60%, 70%, 80%, 90% or 95%. If necessary the percentage homology will be specified. The terms "homology" and "identity" are often used interchangeably. In general, sequences are aligned
20 so that the highest order match is obtained (see, e.g.: *Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part I*, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence*
25 *Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; Carillo *et al.* (1988) *SIAM J Applied Math* 48:1073). By sequence identity, the number of conserved amino acids are determined by standard alignment algorithms programs, and are used with default gap penalties
30 established by each supplier. Substantially homologous nucleic acid molecules would hybridize typically at moderate stringency or at high stringency all along the length of the nucleic acid or or along at least about 70%, 80% or 90% of

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the full length nucleic acid molecule of interest. Also contemplated are nucleic acid molecules that contain degenerate codons in place of codons in the hybridizing nucleic acid molecule.

- Whether any two nucleic acid molecules have nucleotide sequences that
- 5 are at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% "identical" can be determined using known computer algorithms such as the "FAST A" program, using for example, the default parameters as in Pearson *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:2444 (other programs include the GCG program package (Devereux, J., *et al.*, *Nucleic Acids Research* 12(1):387 (1984)), BLASTP,
- 10 BLASTN, FASTA (Atschul, S.F., *et al.*, *J Molec Biol* 215:403 (1990); Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo *et al.* (1988) *SIAM J Applied Math* 48:1073). For example, the BLAST function of the National Center for Biotechnology Information database can be used to determine identity. Other commercially or publicly available programs
- 15 include, DNASTar "MegAlign" program (Madison, WI) and the University of Wisconsin Genetics Computer Group (UWG) "Gap" program (Madison WI)). Percent homology or identity of proteins and/or nucleic acid molecules can be determined, for example, by comparing sequence information using a GAP computer program (*e.g.*, Needleman *et al.* (1970) *J. Mol. Biol.* 48:443, as
- 20 revised by Smith and Waterman ((1981) *Adv. Appl. Math.* 2:482). Briefly, the GAP program defines similarity as the number of aligned symbols (*i.e.*, nucleotides or amino acids) which are similar, divided by the total number of symbols in the shorter of the two sequences. Default parameters for the GAP program can include: (1) a unary comparison matrix (containing a value of 1 for
- 25 identities and 0 for non-identities) and the weighted comparison matrix of Gribskov *et al.* (1986) *Nucl. Acids Res.* 14:6745, as described by Schwartz and Dayhoff, eds., *ATLAS OF PROTEIN SEQUENCE AND STRUCTURE*, National Biomedical Research Foundation, pp. 353-358 (1979); (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no
- 30 penalty for end gaps. Therefore, as used herein, the term "identity" represents a comparison between a test and a reference polypeptide or polynucleotide.

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As used herein, the term at least "90% identical to" refers to percent identities from 90 to 99.99 relative to the reference polypeptides. Identity at a level of 90% or more is indicative of the fact that, assuming for exemplification purposes a test and reference polynucleotide length of 100 amino acids are compared. No more than 10% (i.e., 10 out of 100) amino acids in the test polypeptide differs from that of the reference polypeptides. Similar comparisons can be made between a test and reference polynucleotides. Such differences can be represented as point mutations randomly distributed over the entire length of an amino acid sequence or they can be clustered in one or more locations of varying length up to the maximum allowable, e.g. 10/100 amino acid difference (approximately 90% identity). Differences are defined as nucleic acid or amino acid substitutions, or deletions. At the level of homologies or identities above about 85-90%, the result should be independent of the program and gap parameters set; such high levels of identity can be assessed readily, often without relying on software.

As used herein, primer refers to an oligonucleotide containing two or more deoxyribonucleotides or ribonucleotides, typically more than three, from which synthesis of a primer extension product can be initiated. Experimental conditions conducive to synthesis include the presence of nucleoside triphosphates and an agent for polymerization and extension, such as DNA polymerase, and a suitable buffer, temperature and pH.

As used herein, animals include any animal, such as, but are not limited to, goats, cows, deer, sheep, rodents, pigs and humans. Non-human animals, exclude humans as the contemplated animal. The SPs provided herein are from any source, animal, plant, prokaryotic and fungal. Most CVSP14s are of animal origin, including mammalian origin.

As used herein, genetic therapy involves the transfer of heterologous nucleic acid, such as DNA, into certain cells, target cells, of a mammal, particularly a human, with a disorder or conditions for which such therapy is sought. The nucleic acid, such as DNA, is introduced into the selected target cells in a manner such that the heterologous nucleic acid, such as DNA, is expressed and a therapeutic product encoded thereby is produced.

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Alternatively, the heterologous nucleic acid, such as DNA, can in some manner mediate expression of DNA that encodes the therapeutic product, or it can encode a product, such as a peptide or RNA that in some manner mediates, directly or indirectly, expression of a therapeutic product. Genetic therapy can also be used to deliver nucleic acid encoding a gene product that replaces a defective gene or supplements a gene product produced by the mammal or the cell in which it is introduced. The introduced nucleic acid can encode a therapeutic compound, such as a growth factor inhibitor thereof, or a tumor necrosis factor or inhibitor thereof, such as a receptor therefor, that is not normally produced in the mammalian host or that is not produced in therapeutically effective amounts or at a therapeutically useful time. The heterologous nucleic acid, such as DNA, encoding the therapeutic product can be modified prior to introduction into the cells of the afflicted host in order to enhance or otherwise alter the product or expression thereof. Genetic therapy can also involve delivery of an inhibitor or repressor or other modulator of gene expression.

As used herein, heterologous nucleic acid is nucleic acid that (if DNA encodes RNA) and proteins that are not normally produced *in vivo* by the cell in which it is expressed or that mediates or encodes mediators that alter expression of endogenous nucleic acid, such as DNA, by affecting transcription, translation, or other regulatable biochemical processes. Heterologous nucleic acid, such as DNA, can also be referred to as foreign nucleic acid, such as DNA. Any nucleic acid, such as DNA, that one of skill in the art would recognize or consider as heterologous or foreign to the cell in which is expressed is herein encompassed by heterologous nucleic acid; heterologous nucleic acid includes exogenously added nucleic acid that is also expressed endogenously. Examples of heterologous nucleic acid include, but are not limited to, nucleic acid that encodes traceable marker proteins, such as a protein that confers drug resistance, nucleic acid that encodes therapeutically effective substances, such as anti-cancer agents, enzymes and hormones, and nucleic acid, such as DNA, that encodes other types of proteins, such as antibodies. Antibodies that are encoded by heterologous nucleic acid can be secreted or expressed on the

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surface of the cell in which the heterologous nucleic acid has been introduced. Heterologous nucleic acid is generally not endogenous to the cell into which it is introduced, but has been obtained from another cell or prepared synthetically. Generally, although not necessarily, such nucleic acid encodes RNA and proteins that are not normally produced by the cell in which it is expressed.

5 Hence, herein heterologous nucleic acid or foreign nucleic acid, includes a nucleic acid molecule not present in the exact orientation or position as the counterpart nucleic acid molecule, such as DNA, found in the genome. It can also refer to a nucleic acid molecule from another organism or species (*i.e.*,
10 exogenous).

As used herein, a therapeutically effective product is a product that is encoded by heterologous nucleic acid, typically DNA, that, upon introduction of the nucleic acid into a host, a product is expressed that ameliorates or eliminates the symptoms, manifestations of an inherited or acquired disease or that cures
15 the disease.

As used herein, recitation that a polypeptide consists essentially of the protease domain means that the only SP portion of the polypeptide is a protease domain or a catalytically active portion thereof. The polypeptide can optionally, and generally will, include additional non-SP-derived sequences of amino acids.

20 As used herein, cancer or tumor treatment or agent refers to any therapeutic regimen and/or compound that, when used alone or in combination with other treatments or compounds, can alleviate, reduce, ameliorate, prevent, or place or maintain in a state of remission of clinical symptoms or diagnostic markers associated with deficient angiogenesis.

25 As used herein, domain refers to a portion of a molecule, *e.g.*, proteins or the encoding nucleic acids, that is structurally and/or functionally distinct from other portions of the molecule.

As used herein, protease refers to an enzyme catalyzing hydrolysis of proteins or peptides. It includes the zymogen form and activated forms thereof.
30 For clarity reference to protease refers to all forms, and particular forms will be specifically designated. For purposes herein, the protease domain includes single

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and two chain forms of the protease domain of an SP protein. For CVSP14 the protease domain also includes two chain forms of the protease domain.

As used herein, nucleic acids include DNA, RNA and analogs thereof, including protein nucleic acids (PNA) and mixture thereof. Nucleic acids can be
5 single or double-stranded. When referring to probes or primers, optionally labeled, with a detectable label, such as a fluorescent or radiolabel, single-stranded molecules are contemplated. Such molecules are typically of a length such that their target is statistically unique or of low copy number (typically less than 5, generally less than 3) for probing or priming a library. Generally a probe
10 or primer contains at least 14, 16 or 30 contiguous of sequence complementary to or identical a gene of interest. Probes and primers can be 10, 20, 30, 50, 100 or more nucleic acids long.

As used herein, nucleic acid encoding a fragment or portion of an SP refers to a nucleic acid encoding only the recited fragment or portion of SP, and
15 not the other contiguous portions of the SP.

As used herein, operative linkage of heterologous nucleic to regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences refers to the relationship between such nucleic acid, such as DNA, and such sequences of
20 nucleotides. For example, operative linkage of heterologous DNA to a promoter refers to the physical relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA in reading frame. Thus, operatively linked or operationally associated refers to the
25 functional relationship of nucleic acid, such as DNA, with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is
30 initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA. In order to optimize expression and/or *in vitro* transcription, it can be necessary to remove, add or alter 5' untranslated portions

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of the clones to eliminate extra, potential inappropriate alternative translation initiation (*i.e.*, start) codons or other sequences that can interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, *e.g.*, Kozak *J. Biol. Chem.* 266:19867-5 19870 (1991)) can be inserted immediately 5' of the start codon and can enhance expression. The desirability of (or need for) such modification can be empirically determined.

As used herein, a sequence complementary to at least a portion of an RNA, with reference to antisense oligonucleotides, means a sequence having 10 sufficient complementarity to be able to hybridize with the RNA, generally under moderate or high stringency conditions, forming a stable duplex; in the case of double-stranded SP antisense nucleic acids, a single strand of the duplex DNA (or dsRNA) can thus be tested, or triplex formation can be assayed. The ability to hybridize depends on the degree of complementarity and the length of the 15 antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with a SP encoding RNA it can contain and still form a stable duplex (or triplex, as the case can be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

20 For purposes herein, amino acid substitutions can be made in any of SPs and protease domains thereof provided that the resulting protein exhibits protease activity. Muteins can be made by making conservative amino acid substitutions and also non-conservative amino acid substitutions. For example, amino acid substitutions the desirably alter properties of the proteins can be 25 made. In one embodiment, mutations that prevent degradation of the polypeptide can be made. Many proteases cleave after basic residues, such as R and K; to eliminate such cleavage, the basic residue is replaced with a non-basic residue. Interaction of the protease with an inhibitor can be blocked while retaining catalytic activity by effecting a non-conservative change at the site 30 interaction of the inhibitor with the protease. Receptor binding can be altered without altering catalytic activity.

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Amino acid substitutions contemplated include conservative substitutions, such as those set forth in Table 1, which do not eliminate proteolytic activity. As described herein, substitutions that alter properties of the proteins, such as removal of cleavage sites and other such sites are also contemplated; such

5 substitutions are generally non-conservative, but can be readily effected by those of skill in the art.

Suitable conservative substitutions of amino acids are known to those of skill in this art and can be made generally without altering the biological activity, for example enzymatic activity, of the resulting molecule. Those of skill in this

10 art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson *et al. Molecular Biology of the Gene*, 4th Edition, 1987, The Bejacmin/Cummings Pub. co., p.224). Also included within the definition, is the catalytically active fragment of an SP, particularly a single chain protease

15 portion. Conservative amino acid substitutions are made, for example, in accordance with those set forth in TABLE 1 as follows:

TABLE 1

	Original residue	Conservative substitution
	Ala (A)	Gly; Ser; Abu
20	Arg (R)	Lys, orn
	Asn (N)	Gln; His
	Cys (C)	Ser
	Gln (Q)	Asn
	Glu (E)	Asp
25	Gly (G)	Ala; Pro
	His (H)	Asn; Gln
	Ile (I)	Leu; Val; Met; Nle; Nva
	Leu (L)	Ile; Val; Met; Nle; Nv
	Lys (K)	Arg; Gln; Glu
30	Met (M)	Leu; Tyr; Ile; NLe Val
	Ornithine	Lys; Arg
	Phe (F)	Met; Leu; Tyr
	Ser (S)	Thr
	Thr (T)	Ser
35	Trp (W)	Tyr
	Tyr (Y)	Trp; Phe
	Val (V)	Ile; Leu; Met; Nle; Nv

Other substitutions are also permissible and can be determined empirically or in accord with known conservative substitutions.

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As used herein, Abu is 2-aminobutyric acid; Orn is ornithine.

As used herein, the amino acids, which occur in the various amino acid sequences appearing herein, are identified according to their well-known, three-letter or one-letter abbreviations. The nucleotides, which occur in the various

5 DNA fragments, are designated with the standard single-letter designations used routinely in the art.

As used herein, a probe or primer based on a nucleotide sequence disclosed herein, includes at least 10, 14, typically at least 16 contiguous sequence of nucleotides of SEQ ID No. 5, and probes of at least 30, 50 or 100
10 contiguous sequence of nucleotides of SEQ ID No. 5. The length of the probe or primer for unique hybridization is a function of the complexity of the genome of interest.

As used herein, amelioration of the symptoms of a particular disorder by administration of a particular pharmaceutical composition refers to any lessening,
15 whether permanent or temporary, lasting or transient that can be attributed to or associated with administration of the composition.

As used herein, antisense polynucleotides refer to synthetic sequences of nucleotide bases complementary to mRNA or the sense strand of double-stranded DNA. Admixture of sense and antisense polynucleotides under
20 appropriate conditions leads to the binding of the two molecules, or hybridization. When these polynucleotides bind to (hybridize with) mRNA, inhibition of protein synthesis (translation) occurs. When these polynucleotides bind to double-stranded DNA, inhibition of RNA synthesis (transcription) occurs. The resulting inhibition of translation and/or transcription leads to an inhibition of
25 the synthesis of the protein encoded by the sense strand. Antisense nucleic acid molecule typically contain a sufficient number of nucleotides to specifically bind to a target nucleic acid, generally at least 5 contiguous nucleotides, often at least 14 or 16 or 30 contiguous nucleotides or modified nucleotides complementary to the coding portion of a nucleic acid molecule that encodes a
30 gene of interest, for example, nucleic acid encoding a single chain protease domain of an SP.

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As used herein, an array refers to a collection of elements, such as antibodies, containing three or more members. An addressable array is one in which the members of the array are identifiable, typically by position on a solid phase support. Hence, in general the members of the array are immobilized on discrete identifiable loci on the surface of a solid phase.

As used herein, antibody refers to an immunoglobulin, whether natural or partially or wholly synthetically produced, including any derivative thereof that retains the specific binding ability the antibody. Hence antibody includes any protein having a binding domain that is homologous or substantially homologous to an immunoglobulin binding domain. Antibodies include members of any immunoglobulin claims, including IgG, IgM, IgA, IgD and IgE.

As used herein, antibody fragment refers to any derivative of an antibody that is less than full length, retaining at least a portion of the full-length antibody's specific binding ability. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab)₂, single-chain Fvs (scFV), FV, dsFV diabody and Fd fragments. The fragment can include multiple chains linked together, such as by disulfide bridges. An antibody fragment generally contains at least about 50 amino acids and typically at least 200 amino acids.

As used herein, an Fv antibody fragment is composed of one variable heavy domain (V_H) and one variable light domain linked by noncovalent interactions.

As used herein, a dsFV refers to an Fv with an engineered intermolecular disulfide bond, which stabilizes the V_H-V_L pair.

As used herein, an F(ab)₂ fragment is an antibody fragment that results from digestion of an immunoglobulin with pepsin at pH 4.0-4.5; it can be recombinantly produced to produce the equivalent fragment.

As used herein, Fab fragments is an antibody fragment that results from digestion of an immunoglobulin with papain; it can be recombinantly produced to produce the equivalent fragment.

As used herein, scFVs refer to antibody fragments that contain a variable light chain (V_L) and variable heavy chain (V_H) covalently connected by a polypeptide linker in any order. The linker is of a length such that the two

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variable domains are bridged without substantial interference. Included linkers are (Gly-Ser)_n residues with some Glu or Lys residues dispersed throughout to increase solubility.

As used herein, humanized antibodies refer to antibodies that are
5 modified to include human sequences of amino acids so that administration to a human does not provoke an immune response. Methods for preparation of such antibodies are known. For example, the hybridoma that expresses the monoclonal antibody is altered by recombinant DNA techniques to express an antibody in which the amino acid composition of the non-variable regions is
10 based on human antibodies. Computer programs have been designed to identify such regions.

As used herein, diabodies are dimeric scFV; diabodies typically have shorter peptide linkers than scFVs, and they generally dimerize.

As used herein, production by recombinant means by using recombinant
15 DNA methods means the use of the well known methods of molecular biology for expressing proteins encoded by cloned DNA.

As used herein the term assessing is intended to include quantitative and qualitative determination in the sense of obtaining an absolute value for the activity of an SP, or a domain thereof, present in the
20 sample, and also of obtaining an index, ratio, percentage, visual or other value indicative of the level of the activity. Assessment can be direct or indirect and the chemical species actually detected need not of course be the proteolysis product itself but can for example be a derivative thereof or some further substance.

25 As used herein, biological activity refers to the *in vivo* activities of a compound or physiological responses that result upon *in vivo* administration of a compound, composition or other mixture. Biological activity, thus, encompasses therapeutic effects and pharmaceutical activity of such compounds, compositions and mixtures. Biological activities can be observed in *in vitro*
30 systems designed to test or use such activities. Thus, for purposes herein the biological activity of a luciferase is its oxygenase activity whereby, upon oxidation of a substrate, light is produced.

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As used herein, functional activity refers to a polypeptide or portion thereof that displays one or more activities associated with a full-length (complete) protein. Functional activities include, but are not limited to, biological activity, catalytic or enzymatic activity, antigenicity (ability to bind to or compete
5 with a polypeptide for binding to an anti-polypeptide antibody), immunogenicity, ability to form multimers, the ability to specifically bind to a receptor or ligand for the polypeptide.

As used herein, a conjugate refers to the compounds provided herein that include one or more SPs, including a CVSP14, particularly single chain protease
10 domains thereof, and one or more targeting agents. These conjugates include those produced by recombinant means as fusion proteins, those produced by chemical means, such as by chemical coupling, through, for example, coupling to sulfhydryl groups, and those produced by any other method whereby at least one SP, or a domain thereof, is linked, directly or indirectly via linker(s) to a
15 targeting agent.

As used herein, a targeting agent, is any moiety, such as a protein or effective portion thereof, that provides specific binding of the conjugate to a cell surface receptor, which, can internalize the conjugate or SP portion thereof. A targeting agent can also be one that promotes or facilitates, for example, affinity
20 isolation or purification of the conjugate; attachment of the conjugate to a surface; or detection of the conjugate or complexes containing the conjugate.

As used herein, an antibody conjugate refers to a conjugate in which the targeting agent is an antibody.

As used herein, derivative or analog of a molecule refers to a portion
25 derived from or a modified version of the molecule.

As used herein, an effective amount of a compound for treating a particular disease is an amount that is sufficient to ameliorate, or in some manner reduce the symptoms associated with the disease. Such amount can be administered as a single dosage or can be administered according to a regimen,
30 whereby it is effective. The amount can cure the disease but, typically, is administered in order to ameliorate the symptoms of the disease. Repeated administration can be required to achieve the desired amelioration of symptoms.

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As used herein equivalent, when referring to two sequences of nucleic acids means that the two sequences in question encode the same sequence of amino acids or equivalent proteins. When equivalent is used in referring to two proteins or peptides, it means that the two proteins or peptides have

5 substantially the same amino acid sequence with only amino acid substitutions (such, as but not limited to, conservative changes such as those set forth in Table 1, above) that do not substantially alter the activity or function of the protein or peptide. When equivalent refers to a property, the property does not need to be present to the same extent (*e.g.*, two peptides can exhibit different
10 rates of the same type of enzymatic activity), but the activities are usually substantially the same. Complementary, when referring to two nucleotide sequences, means that the two sequences of nucleotides are capable of hybridizing, typically with less than 25%, 15%, 5% or 0% mismatches between opposed nucleotides. If necessary the percentage of complementarity will be
15 specified. Typically the two molecules are selected such that they will hybridize under conditions of high stringency.

As used herein, an agent that modulates the activity of a protein or expression of a gene or nucleic acid either decreases or increases or otherwise alters the activity of the protein or, in some manner up- or down-regulates or
20 otherwise alters expression of the nucleic acid in a cell.

As used herein, inhibitor of the activity of an SP encompasses any substances that prohibit or decrease production, post-translational modification(s), maturation, or membrane localization of the SP or any substances that interferes with or decreases the proteolytic efficacy of thereof,
25 particularly of a single chain form in an *in vitro* screening assay.

As used herein, a method for treating or preventing neoplastic disease means that any of the symptoms, such as the tumor, metastasis thereof, the vascularization of the tumors or other parameters by which the disease is characterized are reduced, ameliorated, prevented, placed in a state of remission,
30 or maintained in a state of remission. It also means that the hallmarks of neoplastic disease and metastasis can be eliminated, reduced or prevented by the treatment. Non-limiting examples of the hallmarks include uncontrolled

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degradation of the basement membrane and proximal extracellular matrix, migration, division, and organization of the endothelial cells into new functioning capillaries, and the persistence of such functioning capillaries.

As used herein, pharmaceutically acceptable salts, esters or other
5 derivatives of the conjugates include any salts, esters or derivatives that can be readily prepared by those of skill in this art using known methods for such derivatization and that produce compounds that can be administered to animals or humans without substantial toxic effects and that either are pharmaceutically active or are prodrugs.

10 As used herein, a prodrug is a compound that, upon *in vivo* administration, is metabolized or otherwise converted to the biologically, pharmaceutically or therapeutically active form of the compound. To produce a prodrug, the pharmaceutically active compound is modified such that the active compound is regenerated by metabolic processes. The prodrug can be designed
15 to alter the metabolic stability or the transport characteristics of a drug, to mask side effects or toxicity, to improve the flavor of a drug or to alter other characteristics or properties of a drug. By virtue of knowledge of pharmacodynamic processes and drug metabolism *in vivo*, those of skill in this art, once a pharmaceutically active compound is known, can design prodrugs of
20 the compound (see, *e.g.*, Nogrady (1985) *Medicinal Chemistry A Biochemical Approach*, Oxford University Press, New York, pages 388-392).

As used herein, a drug identified by the screening methods provided herein refers to any compound that is a candidate for use as a therapeutic or as a lead compound for the design of a therapeutic. Such compounds can be small
25 molecules, including small organic molecules, peptides, peptide mimetics, antisense molecules or dsRNA, such as RNAi, antibodies, fragments of antibodies, recombinant antibodies and other such compound which can serve as drug candidate or lead compound.

As used herein, a peptidomimetic is a compound that mimics the
30 conformation and certain stereochemical features of the biologically active form of a particular peptide. In general, peptidomimetics are designed to mimic certain desirable properties of a compound, but not the undesirable properties,

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such as flexibility, that lead to a loss of a biologically active conformation and bond breakdown. Peptidomimetics may be prepared from biologically active compounds by replacing certain groups or bonds that contribute to the undesirable properties with bioisosteres. Bioisosteres are known to those of skill in the art. For example the methylene bioisostere CH_2S has been used as an amide replacement in enkephalin analogs (see, e.g., Spatola (1983) pp. 267-357 in Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins, Weistein, Ed. volume 7, Marcel Dekker, New York). Morphine, which can be administered orally, is a compound that is a peptidomimetic of the peptide endorphin. For purposes herein, cyclic peptides are included among peptidomimetics.

As used herein, a promoter region or promoter element refers to a segment of DNA or RNA that controls transcription of the DNA or RNA to which it is operatively linked. The promoter region includes specific sequences that are sufficient for RNA polymerase recognition, binding and transcription initiation. This portion of the promoter region is referred to as the promoter. In addition, the promoter region includes sequences that modulate this recognition, binding and transcription initiation activity of RNA polymerase. These sequences can be *cis* acting or can be responsive to *trans* acting factors. Promoters, depending upon the nature of the regulation, can be constitutive or regulated. Exemplary promoters contemplated for use in prokaryotes include the bacteriophage T7 and T3 promoters.

As used herein, a receptor refers to a molecule that has an affinity for a given ligand. Receptors can be naturally-occurring or synthetic molecules. Receptors can also be referred to in the art as anti-ligands. As used herein, the receptor and anti-ligand are interchangeable. Receptors can be used in their unaltered state or as aggregates with other species. Receptors can be attached, covalently or noncovalently, or in physical contact with, to a binding member, either directly or indirectly via a specific binding substance or linker. Examples of receptors, include, but are not limited to: antibodies, cell membrane receptors, surface receptors and internalizing receptors, monoclonal antibodies and antisera reactive with specific antigenic determinants [such as on viruses, cells, or other

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materials], drugs, polynucleotides, nucleic acids, peptides, cofactors, lectins, sugars, polysaccharides, cells, cellular membranes, and organelles.

Examples of receptors and applications using such receptors, include but are not restricted to:

5 a) enzymes: specific transport proteins or enzymes essential to survival of microorganisms, which could serve as targets for antibiotic [ligand] selection;

 b) antibodies: identification of a ligand-binding site on the antibody molecule that combines with the epitope of an antigen of interest can be investigated; determination of a sequence that mimics an antigenic epitope can
10 lead to the development of vaccines of which the immunogen is based on one or more of such sequences or lead to the development of related diagnostic agents or compounds useful in therapeutic treatments such as for auto-immune diseases

 c) nucleic acids: identification of ligand, such as protein or RNA, binding sites;

15 d) catalytic polypeptides: polymers, including polypeptides, that are capable of promoting a chemical reaction involving the conversion of one or more reactants to one or more products; such polypeptides generally include a binding site specific for at least one reactant or reaction intermediate and an active functionality proximate to the binding site, in which the functionality is
20 capable of chemically modifying the bound reactant (see, *e.g.*, U.S. Patent No. 5,215,899);

 e) hormone receptors: determination of the ligands that bind with high affinity to a receptor is useful in the development of hormone replacement therapies; for example, identification of ligands that bind to such receptors can
25 lead to the development of drugs to control blood pressure; and

 f) opiate receptors: determination of ligands that bind to the opiate receptors in the brain is useful in the development of less-addictive replacements for morphine and related drugs.

 As used herein, sample refers to anything which can contain an analyte
30 for which an analyte assay is desired. The sample can be a biological sample, such as a biological fluid or a biological tissue. Examples of biological fluids include urine, blood, plasma, serum, saliva, semen, stool, sputum, cerebral spinal

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fluid, tears, mucus, amniotic fluid or the like. Biological tissues are aggregate of cells, usually of a particular kind together with their intercellular substance that form one of the structural materials of a human, animal, plant, bacterial, fungal or viral structure, including connective, epithelium, muscle and nerve tissues.

- 5 Examples of biological tissues also include organs, tumors, lymph nodes, arteries and individual cell(s).

As used herein: stringency of hybridization in determining percentage mismatch is as follows:

- 10 1) high stringency: 0.1 x SSPE, 0.1% SDS, 65°C
2) medium stringency: 0.2 x SSPE, 0.1% SDS, 50°C
3) low stringency: 1.0 x SSPE, 0.1% SDS, 50°C

- Those of skill in this art know that the washing step selects for stable hybrids and also know the ingredients of SSPE (see, e.g., Sambrook, E.F. Fritsch, T. Maniatis, in: *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press (1989), vol. 3, p. B.13, see, also, numerous catalogs
15 that describe commonly used laboratory solutions). SSPE is pH 7.4 phosphate-buffered 0.18 M NaCl. Further, those of skill in the art recognize that the stability of hybrids is determined by T_m , which is a function of the sodium ion concentration and temperature ($T_m = 81.5^\circ \text{C} - 16.6(\log_{10}[\text{Na}^+]) + 0.41(\% \text{G} + \text{C}) - 600/l$), so that the only parameters in the wash conditions critical to hybrid
20 stability are sodium ion concentration in the SSPE (or SSC) and temperature.

- It is understood that equivalent stringencies can be achieved using alternative buffers, salts and temperatures. By way of example and not limitation, procedures using conditions of low stringency are as follows (see also
25 Shilo and Weinberg, *Proc. Natl. Acad. Sci. USA* 78:6789-6792 (1981)): Filters containing DNA are pretreated for 6 hours at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 µg/ml denatured salmon sperm DNA (10X SSC is 1.5 M sodium chloride, and 0.15 M sodium citrate, adjusted to a pH of 7).

- 30 Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and $5-20 \times 10^6$ cpm ^{32}P -labeled probe is

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used. Filters are incubated in hybridization mixture for 18-20 hours at 40°C, and then washed for 1.5 hours at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 hours at 60°C. Filters are
5 blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film. Other conditions of low stringency which can be used are well known in the art (e.g., as employed for cross-species hybridizations).

By way of example and not way of limitation, procedures using
10 conditions of moderate stringency include, for example, but are not limited to, procedures using such conditions of moderate stringency are as follows: Filters containing DNA are pretreated for 6 hours at 55°C in a solution containing 6X SSC, 5X Denhart's solution, 0.5% SDS and 100 µg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution and 5-20 X 10⁶ cpm
15 ³²P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 hours at 55°C, and then washed twice for 30 minutes at 60°C in a solution containing 1X SSC and 0.1% SDS. Filters are blotted dry and exposed for autoradiography. Other conditions of moderate stringency which can be used are well-known in the art. Washing of filters is done at 37°C for 1 hour in a
20 solution containing 2X SSC, 0.1% SDS.

By way of example and not way of limitation, procedures using conditions of high stringency are as follows: Prehybridization of filters containing DNA is carried out for 8 hours to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA,
25 and 500 µg/ml denatured salmon sperm DNA. Filters are hybridized for 48 hours at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10⁶ cpm of ³²P-labeled probe. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45
30 minutes before autoradiography. Other conditions of high stringency which can be used are well known in the art.

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The term substantially identical or homologous or similar varies with the context as understood by those skilled in the relevant art and generally means at least 60% or 70%, preferably means at least 80%, more preferably at least 90%, and most preferably at least 95% identity.

5 As used herein, substantially identical to a product means sufficiently similar so that the property of interest is sufficiently unchanged so that the substantially identical product can be used in place of the product.

 As used herein, substantially pure means sufficiently homogeneous to appear free of readily detectable impurities as determined by standard methods
10 of analysis, such as thin layer chromatography (TLC), gel electrophoresis and high performance liquid chromatography (HPLC), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the
15 compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound can, however, be a mixture of stereoisomers or isomers. In such instances, further purification might increase the specific activity of the compound.

 As used herein, target cell refers to a cell that expresses an SP *in vivo*.

20 As used herein, test substance (or test compound) refers to a chemically defined compound (*e.g.*, organic molecules, inorganic molecules, organic/inorganic molecules, proteins, peptides, nucleic acids, oligonucleotides, lipids, polysaccharides, saccharides, or hybrids among these molecules such as glycoproteins, etc.) or mixtures of compounds (*e.g.*, a library of test compounds,
25 natural extracts or culture supernatants, etc.) whose effect on an SP, particularly a single chain form that includes the protease domain or a sufficient portion thereof for activity, as determined by an *in vitro* method, such as the assays provided herein.

 As used herein, the terms a therapeutic agent, therapeutic regimen,
30 radioprotectant, chemotherapeutic mean conventional drugs and drug therapies, including vaccines, which are known to those skilled in the art. Radiotherapeutic agents are well known in the art.

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As used herein, treatment means any manner in which the symptoms of a condition, disorder or disease are ameliorated or otherwise beneficially altered. Treatment also encompasses any pharmaceutical use of the compositions herein.

As used herein, vector (or plasmid) refers to discrete elements that are
5 used to introduce heterologous nucleic acid into cells for either expression or replication thereof. The vectors typically remain episomal, but can be designed to effect integration of a gene or portion thereof into a chromosome of the genome. Also contemplated are vectors that are artificial chromosomes, such as yeast artificial chromosomes and mammalian artificial chromosomes. Selection
10 and use of such vehicles are well known to those of skill in the art. An expression vector includes vectors capable of expressing DNA that is operatively linked with regulatory sequences, such as promoter regions, that are capable of effecting expression of such DNA fragments. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a phage,
15 recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the cloned DNA. Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome.

20 As used herein, protein binding sequence refers to a protein or peptide sequence that is capable of specific binding to other protein or peptide sequences generally, to a set of protein or peptide sequences or to a particular protein or peptide sequence.

As used herein, epitope tag refers to a short stretch of amino acid
25 residues corresponding to an epitope to facilitate subsequent biochemical and immunological analysis of the epitope tagged protein or peptide. Epitope tagging is achieved by including the sequence of the epitope tag to the protein-encoding sequence in an appropriate expression vector. Epitope tagged proteins can be affinity purified using highly specific antibodies raised against the tags.

30 As used herein, metal binding sequence refers to a protein or peptide sequence that is capable of specific binding to metal ions generally, to a set of metal ions or to a particular metal ion.

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As used herein, a combination refers to any association between two or among more items.

As used herein, a composition refers to a any mixture. It can be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any
5 combination thereof.

As used herein, fluid refers to any composition that can flow. Fluids thus encompass compositions that are in the form of semi-solids, pastes, solutions, aqueous mixtures, gels, lotions, creams and other such compositions.

As used herein, a cellular extract refers to a preparation or fraction which
10 is made from a lysed or disrupted cell.

As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences involved in the association of a protein alone or with its associated substrates, binding partners, etc. An example of randomly selected agents is the use a chemical library or a
15 peptide combinatorial library, or a growth broth of an organism or conditioned medium.

As used herein, an agent is said to be rationally selected or designed when the agent is chosen on a non-random basis which takes into account the sequence of the target site and/or its conformation in connection with the
20 agent's action. As described in the Examples, there are proposed binding sites for serine protease and (catalytic) sites in the protein having SEQ ID NO:3 or SEQ ID NO:4. Agents can be rationally selected or rationally designed by utilizing the peptide sequences that make up these sites. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is
25 identical to the ATP or calmodulin binding sites or domains.

For clarity of disclosure, and not by way of limitation, the detailed description is divided into the subsections that follow.

B. CVSP14 polypeptides, muteins, derivatives and analogs thereof
SPs

30 The serine proteases (SPs) are a family of proteins found in mammals and also other species. SPs that share a number of common structural features as described herein. The proteolytic domains share sequence homology including

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conserved His, Asp, and Ser residues necessary for catalytic activity that are present in conserved motifs. These SPs are synthesized as zymogens, and activated to two chain forms by specific cleavage.

The SP family can be target for therapeutic intervention and also can
5 serve as diagnostic markers for tumor initiation, development, growth and/or progression. As discussed, members of this family are involved in proteolytic processes that are implicated in tumor development, growth and/or progression. This implication is based upon their functions as proteolytic enzymes in extracellular matrix degradation and remodelling and growth and pro-angiogenic
10 factor activation. In addition, their levels of expression or level of activation or their apparent activity resulting from substrate levels or alterations in substrates and levels thereof differs in tumor cells and non-tumor cells in the same tissue. Hence, protocols and treatments that alter their activity, such as their proteolytic activities and roles in signal transduction, and/or their expression, such as by
15 contacting them with a compound that modulates their activity and/or expression, could impact tumor development, growth and/or progression. Also, in some instances, the level of activation and/or expression can be altered in tumors, such as pancreas, stomach, uterus, lung, colon and cervical cancers, and also breast, prostate or leukemias. The SP, thus, can serve as a diagnostic
20 marker for tumors.

In other instances the SP protein can exhibit altered activity by virtue of a change in activity or expression of a co-factor therefor or a substrate therefor. Detection of the SPs, particularly the protease domains, in body fluids, such as serum, blood, saliva, cerebral spinal fluid, synovial fluid and interstitial fluids,
25 urine, sweat and other such fluids and secretions, can serve as a diagnostic tumor marker. In particular, detection of higher levels of such polypeptides in a subject compared to a subject known not to have any neoplastic disease or compared to earlier samples from the same subject, can be indicative of neoplastic disease in the subject.

30 Provided is a family member designated CVSP14. It is shown herein, that the CVSP14s provided herein are serine proteases that are expressed and/or activated in certain tumors; hence their activation or expression can serve as a

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diagnostic marker for tumor development, growth and/or progression. The CVSP14 is also provided for use as a drug target and used in screening assays, including those exemplified herein. It is shown herein that the single chain proteolytic domain can function *in vitro* and, hence is useful in *in vitro* assays for
5 identifying agents that modulate the activity of members of this family. In addition the two-chain form or the full-length or truncated forms thereof, such as forms in which the signal peptide is removed can also be used in such assays.

In certain embodiments, the CVSP14 polypeptide is detectable in a body fluid at a level that differs from its level in body fluids in a subject not having a
10 tumor. In other embodiments, the polypeptide is present in a tumor; and a substrate or cofactor for the polypeptide is expressed at levels that differ from its level of expression in a non-tumor cell in the same type of tissue.

CVSP14

Provided are substantially purified CVSP14 zymogens, activated two
15 chain forms, single chain protease domains and two chain protease domains. A full-length CVSP14 polypeptide, including the signal sequence, is set forth in SEQ ID Nos. 12 and 13. The signal sequence can be cleaved upon expression or prior to expression.

Also provided is a substantially purified protein including a sequence of
20 amino acids that has at least 60%, 70%, 80%, 90% or about 95%, identity to the CVSP14 where the percentage identity is determined using standard algorithms and gap penalties that maximize the percentage identity. A human CVSP14 polypeptide is exemplified, although other mammalia CVSP14 polypeptides are contemplated. Splice variants of the CVSP14, particularly
25 those with a proteolytically active protease domain, are contemplated herein.

In other embodiments, substantially purified polypeptides that include a protease domain of a CVSP14 polypeptide or a catalytically active portion thereof, but that do not include the entire sequence of amino acids set forth in SEQ ID No. 13 are provided. Among these are polypeptides that include a
30 sequence of amino acids that has at least 60%, 70%, 80%, 90%, 95% or 100% sequence identity to SEQ ID No. 6.

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Provided are substantially purified CVSP14 polypeptides and functional domains thereof, including catalytically active domains and portions, that have at least about 60%, 70%, 80%, 90% or about 95% sequence identity with a protease domain that includes the sequence of amino acids set forth in SEQ ID No. 6 or a catalytically active portion thereof or with a protease that includes the sequence of amino acids set forth in SEQ ID No. 13 and domains thereof.

With reference to SEQ ID No. 6, the protease activation cleavage site is between R₅₅ and I₅₆; the catalytic triad based upon homology is H₉₈, D₁₄₆, S₂₄₄; there is a potential N-glycosylation site at N₁₀₈VT; Cys pairing is predicted to be between C₃₇-C₁₆₆, which links the protease domain to the remainder of the polypeptide), C₁₈₀-C₂₅₀, C₂₁₁-C₂₂₉ and C₂₄₀-C₂₆₉. Hence C₁₆₆ is a free Cys in the protease domain, which also can be provided as a two chain molecule. It is shown herein, however, that the single chain form is proteolytically active.

Also provided are polypeptides that are encoded by the nucleic acid molecules provided herein. Included among those polypeptides are the CVSP14 protease domain or a polypeptide with amino acid changes such that the specificity and protease activity is not eliminated and is retained at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or remains substantially unchanged. In particular, a substantially purified mammalian SP protein is provided that includes a serine protease catalytic domain and can additionally include other domains. The CVSP14 can form homodimers and can also form heterodimers with some other protein, such as a membrane-bound protein.

The domains, fragments, derivatives or analogs of a CVSP14 that are functionally active are capable of exhibiting one or more functional activities associated with the CVSP14 polypeptide, such as serine protease activity, immunogenicity and antigenicity, are provided.

Antigenic epitopes that contain at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 40, 50, and typically 10-15 amino acids of the CVSP14 polypeptide are provided. These antigenic epitopes are used, for example, to raise antibodies. Antibodies specific for each epitope or combinations thereof and for single and two-chain forms are also provided.

Mutins and derivatives of CVSP14 polypeptides

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Full-length CVSP14, zymogen and activated forms thereof and CVSP14 protease domains, portions thereof, and muteins and derivatives of such polypeptides are provided. Among the derivatives are those based on animal CVSP14s, including, but are not limited to, rodent, such as mouse and rat; fowl, 5 such as chicken; ruminants, such as goats, cows, deer, sheep; ovine, such as pigs; and humans. For example, CVSP14 derivatives can be made by altering their sequences by substitutions, additions or deletions. CVSP14 derivatives include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of CVSP14, including altered 10 sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence can be 15 selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include 20 arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid (see, *e.g.*, Table 1). Muteins of the CVSP14 or a domain thereof, such as a protease domain, in which up to about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90% or 95% of the amino acids are replaced with another amino acid are provided. Generally such 25 muteins retain at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of the protease activity the unmutated protein.

Muteins in which one or more of the Cys residues, particularly, a residue that is paired in the activated two form, but unpaired in the protease domain alone (*i.e.*, the Cys at residue position 26 (see SEQ ID Nos. 5 and 6) in the 30 protease domain), is/are replaced with any amino acid, typically, although not necessarily, a conservative amino acid residue, such as Ser, are contemplated. Muteins of CVSP14, particularly those in which Cys residues, such as the Cys in

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the single chain protease domain, is replaced with another amino acid that does not eliminate the activity, are provided.

Muteins of the protein are also provided in which amino acids are replaced with other amino acids. Among the muteins are those in which the Cys residues, is/are replaced typically although not necessarily, with a conservative amino acid residues, such as a serine. Such muteins are also provided herein. Muteins in which 10%, 20%, 30%, 35%, 40%, 45%, 50% or more of the amino acids are replaced but the resulting polypeptide retains at least about 10%, 20%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 95% of the catalytic activity as the unmodified form for the same substrate.

Protease domains

Isolated, substantially pure proteases that include the protease domains or catalytically active portions thereof as single chain forms of SPs are provided. The protease domains can be included in a longer protein, and such longer protein is optionally the CVSP14 zymogen. Provided herein are isolated substantially pure single polypeptides that contain the protease domain of a CVSP14 as a single chain. The CVSP14 provided herein is expressed or activated by or in tumor cells, typically at a level that differs from the level in which they are expressed by the non-tumor cell of the same type. Hence, for example, if the SP is expressed by a prostate or ovarian tumor cell, to be of interest herein with respect to ovarian or prostate cancer, it an expression, extent of activation or activity that is different from that in non-tumor cells. CVSP14 is expressed in lung, colon, prostate, breast, uterine, ovarian and other tumor cells.

SP protease domains include the single chain protease domains of CVSP14. Provided are the protease domains or proteins that include a portion of an SP that is the protease domain of any SP, particularly a CVSP14. The protein can also include other non-SP sequences of amino acids, but includes the protease domain or a sufficient portion thereof to exhibit catalytic activity in any *in vitro* assay that assess such protease activity, such as any provided herein. Also provided are two chain activated forms of the full length protease and also two chain forms of the protease domain.

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In an embodiment, the substantially purified SP protease is encoded by a nucleic acid that hybridizes to the a nucleic acid molecule containing the protease domain encoded by the nucleotide sequence set forth in SEQ. ID No. 5 under at least moderate, generally high, stringency conditions, such that the protease domain encoding nucleic acid thereof hybridizes along its full length or along at least about 70%, 80% or 90% of the full length. In other embodiments the substantially purified SP protease is a single chain polypeptide that includes substantially the sequence of amino acids set forth in SEQ ID No. 6, or a catalytically active portion thereof.

10 In particular, exemplary protease domains include at least a sufficient portion of sequences of amino acids set forth in SEQ ID No. 6 (encoded by nucleotides in SEQ ID No. 5) to exhibit protease activity in an assay provided herein.

The signal peptide (amino acids 1-25 of SEQ ID No. 13) is also provided.

15 In addition the mature CVSP15 polypeptide with the signal sequence removed is provided.

As described below, all forms of the CVSP14, including the pro-polypeptide with the signal sequence, the mature polypeptide and catalytically active portions thereof, the protease domains and catalytically active portions thereof, two-chain and single chain forms of any of these proteins are provided herein and can be used in the screening assays and for preparing antibodies specific therefore. The expression, quantity and/or activation of the protein in tumor cells and body fluids can be diagnostic of disease or its absence.

25 **Nucleic acid molecules, vectors and plasmids, cells and expression of CVSP14 polyptides**

Nucleic acid molecules

Due to the degeneracy of nucleotide coding sequences, other nucleic sequences which encode substantially the same amino acid sequence as a CVSP14 gene can be used. These include but are not limited to nucleotide sequences comprising all or portions of CVSP14 genes that are altered by the substitution of different codons that encode the amino acid residue within the sequence, thus producing a silent change.

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Also provided are nucleic acid molecules that hybridize to the above-noted sequences of nucleotides encoding CVSP14 at least at low stringency, at moderate stringency, and/or at high stringency, and that encode the protease domain and/or the full length protein or other domains of a CVSP14 or a splice variant or allelic variant thereof. Generally the molecules hybridize under such conditions along their full length (or along at least about 70%, 80% or 90% of the full length) for at least one domain and encode at least one domain, such as the protease domain, of the polypeptide. In particular, such nucleic acid molecules include any isolated nucleic fragment that encodes at least one domain of a serine protease, that (1) contains a sequence of nucleotides that encodes the protease or a functionally active, such as catalytically active, domain thereof, and (2) is selected from among:

- (a) a sequence of nucleotides that encodes the protease or a domain thereof includes a sequence of nucleotides set forth in SEQ ID Nos. 5 or 12;
- (b) a sequence of nucleotides that encodes such portion or the full length protease and hybridizes under conditions of moderate or high stringency, generally to nucleic acid that is complementary to a mRNA transcript present in a mammalian cell that encodes such protein or fragment thereof;
- (c) a sequence of nucleotides that encodes a serine protease or domain thereof that includes a sequence of amino acids encoded by such portion or the full length open reading frame;
- (d) a sequence of nucleotides that encodes the serine protease that includes a sequence of amino acids encoded by a sequence of nucleotides that encodes such subunit and hybridizes under conditions of high stringency to DNA that is complementary to the mRNA transcript;

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(e) a sequence of nucleotides that encodes a splice variant of any of (a)-(d); and

(f) a sequence of nucleotides that includes degenerate codons of all or a portion of any of (a)-(e).

5 The isolated nucleic acid fragment is DNA, including genomic or cDNA, or is RNA, or can include other components, such as protein nucleic acid. The isolated nucleic acid can include additional components, such as heterologous or native promoters, and other transcriptional and translational regulatory sequences, these genes can be linked to other genes, such as reporter genes or
10 other indicator genes or genes that encode indicators.

The CVS14s provided herein are encoded by a nucleic acid that includes sequence encoding a protease domain that exhibits proteolytic activity and that hybridizes to a nucleic acid molecule including the sequence of nucleotides set forth in SEQ ID No. 5, typically under moderate, generally under high stringency,
15 conditions and generally along the full length of the protease domain or along at least about 70%, 80% or 90% of the full length. Splice variants are also provided herein.

In a specific embodiment, a nucleic acid that encodes a CVSP, designated CVSP14 is provided. In particular, the nucleic acid includes the sequence of
20 nucleotides set forth in SEQ ID No. 5 or a portion thereof that encodes a catalytically active polypeptide. Also provided are nucleic acid molecules that hybridize under conditions of at least low stringency, generally moderate stringency, more typically high stringency to the SEQ ID No. 5 or degenerates thereof.

25 In one embodiment, the isolated nucleic acid fragment hybridizes to a nucleic acid molecule containing the nucleotide sequence set forth in SEQ ID No: 5 (or degenerates thereof) under high stringency conditions, in one embodiments contains the sequence of nucleotides set forth in SEQ ID Nos. 5 and 6). A full-length CVSP14 is set forth in SEQ ID No. 13 and is encoded by SEQ ID No. 12
30 or degenerates thereof.

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Also contemplated are nucleic acid molecules that encode a single chain SP protease that have proteolytic activity in an *in vitro* proteolysis assay and that have at least 60%, 70%, 80%, 85%, 90% or 95% sequence identity with the full length of a protease domain of a CVSP14 polypeptide, or that hybridize
5 along their full length or along at least about 70%, 80% or 90% of the full length to a nucleic acids that encode a protease domain, particularly under conditions of moderate, generally high, stringency. As above, the encoded polypeptides contain the protease as a single chain.

The isolated nucleic acids can contain least 10 nucleotides, 25
10 nucleotides, 50 nucleotides, 100 nucleotides, 150 nucleotides, or 200 nucleotides or more contiguous nucleotides of a CVSP14-encoding sequence, or a full-length SP coding sequence. In another embodiment, the nucleic acids are smaller than 35, 200 or 500 nucleotides in length. Nucleic acids that hybridize to or are complementary to a CVSP14-encoding nucleic acid molecule can be
15 single or double-stranded. For example, nucleic acids are provided that include a sequence complementary to (specifically are the inverse complement of) at least 10, 25, 50, 100, or 200 nucleotides or the entire coding region of a CVSP14 encoding nucleic acid, particularly the protease domain thereof. For CVSP14 the full-length protein or a domain or active fragment thereof is also provided.

20 For each of the nucleic acid molecules, the nucleic acid can be DNA or RNA or PNA or other nucleic acid analogs or can include non-natural nucleotide bases. Also provided are isolated nucleic acid molecules that include a sequence of nucleotides complementary to the nucleotide sequence encoding an SP.

Probes, primers, antisense oligonucleotides and dsRNA

25 Also provided are fragments thereof or oligonucleotides that can be used as probes or primers and that contain at least about 10, 14, 16 nucleotides, generally less than 1000 or less than or equal to 100, set forth in SEQ ID No. 5 (or the complement thereof); or contain at least about 30 nucleotides (or the complement thereof) or contain oligonucleotides that hybridize along their full
30 length or along at least about 70%, 80% or 90% of the full length to any such fragments or oligonucleotides. The length of the fragments are a function of the purpose for which they are used and/or the complexity of the genome of

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interest. Generally probes and primers contain less than about 500, 150, 100 nucleotides.

Probes and primers derived from the nucleic acid molecules are provided, Such probes and primers contain at least 8, 14, 16, 30, 100 or more contiguous
5 nucleotides with identity to contiguous nucleotides of a CVSP14. The probes and primers are optionally labelled with a detectable label, such as a radiolabel or a fluorescent tag, or can be mass differentiated for detection by mass spectrometry or other means.

Also provided is an isolated nucleic acid molecule that includes the
10 sequence of molecules that is complementary to the nucleotide sequence encoding CVSP14 or the portion thereof. Double-stranded RNA (dsRNA), such as RNAi is also provided.

Plasmids, vectors and cells

Plasmids and vectors containing the nucleic acid molecules are also
15 provided. Cells containing the vectors, including cells that express the encoded proteins are provided. The cell can be a bacterial cell, a yeast cell, a fungal cell, a plant cell, an insect cell or an animal cell. Methods for producing an SP or single chain form of the protease domain thereof by, for example, growing the cell under conditions whereby the encoded SP is expressed by the cell, and
20 recovering the expressed protein, are provided herein. As noted, for CVSP14, the full-length zymogens and activated proteins and activated (two chain) protease and single chain protease domains are provided.

As discussed below, the CVSP14 polypeptide, and catalytically active portions thereof, can be expressed as a secreted protein using the native signal
25 sequence or a heterologous signal. Alternatively, as exemplified, the protein can be expressed as inclusion bodies in the cytoplasm and isolated therefrom. The resulting protein can be treated to refold (see, *e.g.*, EXAMPLE 1). It is shown herein that active protease domain can be produced by expression in inclusion bodies, isolation therefrom and denaturation followed by refolding.

C. Tumor specificity and tissue expression profiles

Each SP has a characteristic tissue expression profile; the SPs in particular, although not exclusively expressed or activated in tumors, exhibit characteristic tumor tissue expression or activation profiles. In some instances, SPs can have different activity in a tumor cell from a non-tumor cell by virtue of a change in a substrate or cofactor therefor or other factor that would alter the apparent functional activity of the SP. Hence each can serve as a diagnostic marker for particular tumors, by virtue of a level of activity and/or expression or function in a subject (i.e. a mammal, particularly a human) with neoplastic disease, compared to a subject or subjects that do not have the neoplastic disease. In addition, detection of activity (and/or expression) in a particular tissue can be indicative of neoplastic disease.

Circulating SPs in body fluids can be indicative of neoplastic disease. Secreted CVSP14 or activated CVSP14 is indicative of neoplastic disease. Also, by virtue of the activity and/or expression profiles of each, they can serve as therapeutic targets, such as by administration of modulators of the activity thereof, or, as by administration of a prodrug specifically activated by one of the SPs.

Tissue expression profiles**CVSP14**

The CVSP14 is expressed at high levels in an androgen-independent tumor cell line. The CVSP14 transcript was detected in normal kidney samples. CVSP14 signals were diminished in all the matched kidney tumor samples. Weak signals were detected in all three pairs of prostate normal/tumor cDNA samples. Weak signals were also detected in 3 of 9 normal breast samples. A weak signal was also detected in one of the 7 uterine tumors, but not in their normal tissue counterparts. Weak signals were also detected in two of the three normal lung tissue samples, but not in their matched tumor samples. Very weak signals can be seen in cDNA samples from various tumor cell lines, including HeLa cells, Burkitt's lymphoma Daudi cells, chronic myelogenous leukemia K562, promyelocytic leukemia HL-60 cells, melanoma G361 cells, lung carcinoma A549

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cells, lymphoblastic leukemia MOLT-4 and colorectal adenocarcinoma SW480 cells.

Hence expression in certain cells, such as prostate cancer, can serve as a tumor marker; whereas in other tissues, such as kidney, the absence of expression or activation, can serve as a tumor marker.

D. Identification and isolation of SP protein genes

The SP polypeptides, including CVSP14 polypeptides, or domains thereof, can be obtained by methods well known in the art for protein purification and recombinant protein expression. Any method known to those of skill in the art for identification of nucleic acids that encode desired genes can be used. Any method available in the art can be used to obtain a full length (*i.e.*, encompassing the entire coding region) cDNA or genomic DNA clone encoding an SP protein. In particular, the polymerase chain reaction (PCR) can be used to amplify a sequence identified as being differentially expressed or encoding proteins activated at different levels in tumor and non-tumor cells or tissues, *e.g.*, nucleic acids encoding a CVSP14 polypeptide (SEQ. NOs: 5, 6, 12 and 13), in a genomic or cDNA library. Oligonucleotide primers that hybridize to sequences at the 3' and 5' termini of the identified sequences can be used as primers to amplify by PCR sequences from a nucleic acid sample (RNA or DNA), typically a cDNA library, from an appropriate source (*e.g.*, tumor or cancer tissue).

PCR can be carried out, *e.g.*, by use of a Perkin-Elmer Cetus thermal cyclor and Taq polymerase (Gene Amp™). The DNA being amplified can include mRNA or cDNA or genomic DNA from any eukaryotic species. One can choose to synthesize several different degenerate primers, for use in the PCR reactions. It is also possible to vary the stringency of hybridization conditions used in priming the PCR reactions, to amplify nucleic acid homologs (*e.g.*, to obtain *SP protein* sequences from species other than humans or to obtain human sequences with homology to CVSP14 polypeptide) by allowing for greater or lesser degrees of nucleotide sequence similarity between the known nucleotide sequence and the nucleic acid homolog being isolated. For cross species hybridization, low or moderate stringency conditions are used. For same species

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hybridization, moderately or high stringency conditions generally are used. After successful amplification of the nucleic acid containing all or a portion of the identified SP protein sequence or of a nucleic acid encoding all or a portion of an SP protein homolog, that segment can be molecularly cloned and sequenced, and used as a probe to isolate a complete cDNA or genomic clone. This, in turn, permits the determination of the gene's complete nucleotide sequence, the analysis of its expression, and the production of its protein product for functional analysis. Once the nucleotide sequence is determined, an open reading frame encoding the SP protein gene protein product can be determined by any method well known in the art for determining open reading frames, for example, using publicly available computer programs for nucleotide sequence analysis. Once an open reading frame is defined, it is routine to determine the amino acid sequence of the protein encoded by the open reading frame. In this way, the nucleotide sequences of the entire SP protein genes as well as the amino acid sequences of SP protein proteins and analogs can be identified.

Any eukaryotic cell potentially can serve as the nucleic acid source for the molecular cloning of the SP protein gene. The nucleic acids can be isolated from vertebrate, mammalian, human, porcine, bovine, feline, avian, equine, canine, as well as additional primate sources, insects, plants, etc. The DNA can be obtained by standard procedures known in the art from cloned DNA (*e.g.*, a DNA "library"), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell (see, for example, Sambrook et al., 1989, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, *DNA Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K. Vol. I, II). Clones derived from genomic DNA can contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA contains only exon sequences. Whatever the source, the gene should be molecularly cloned into a suitable vector for propagation of the gene.

In the molecular cloning of the gene from genomic DNA, DNA fragments are generated, some of which encode the desired gene. The DNA can be cleaved at specific sites using various restriction enzymes. Alternatively, one

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can use DNase in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, for example, by sonication. The linear DNA fragments can then be separated according to size by standard techniques, including but not limited to, agarose and polyacrylamide gel electrophoresis and
5 column chromatography.

Once the DNA fragments are generated, identification of the specific DNA fragment containing the desired gene can be accomplished in a number of ways. For example, a portion of the SP protein (of any species) gene (*e.g.*, a PCR amplification product obtained as described above or an oligonucleotide having a
10 sequence of a portion of the known nucleotide sequence) or its specific RNA, or a fragment thereof be purified and labeled, and the generated DNA fragments can be screened by nucleic acid hybridization to the labeled probe (Benton and Davis, *Science* 196:180 (1977); Grunstein and Hogness, *Proc. Natl. Acad. Sci. U.S.A.* 72:3961 (1975)). Those DNA fragments with substantial homology to
15 the probe hybridize. It is also possible to identify the appropriate fragment by restriction enzyme digestion(s) and comparison of fragment sizes with those expected according to a known restriction map if such is available or by DNA sequence analysis and comparison to the known nucleotide sequence of SP protein. Further selection can be carried out on the basis of the properties of the
20 gene. Alternatively, the presence of the gene can be detected by assays based on the physical, chemical, or immunological properties of its expressed product. For example, cDNA clones, or DNA clones which hybrid-select the proper mRNA, can be selected which produce a protein that, *e.g.*, has similar or identical electrophoretic migration, isoelectric focusing behavior, proteolytic
25 digestion maps, antigenic properties, serine protease activity. If an anti-SP protein antibody is available, the protein can be identified by binding of labeled antibody to the putatively SP protein synthesizing clones, in an ELISA (enzyme-linked immunosorbent assay)-type procedure.

Alternatives to isolating the CVSP14 polypeptide genomic DNA include,
30 but are not limited to, chemically synthesizing the gene sequence from a known sequence or making cDNA to the mRNA that encodes the SP protein. For example, RNA for cDNA cloning of the SP protein gene can be isolated from

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- cells expressing the protein. The identified and isolated nucleic acids can then be inserted into an appropriate cloning vector. A large number of vector-host systems known in the art can be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be
- 5 compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as pBR322 or pUC plasmid derivatives or the Bluescript vector (Stratagene, La Jolla, CA). The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini.
- 10 If the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules can be enzymatically modified. Alternatively, any site desired can be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers can comprise specific chemically synthesized oligonucleotides encoding restriction
- 15 endonuclease recognition sequences. In an alternative method, the cleaved vector and SP protein gene can be modified by homopolymeric tailing. Recombinant molecules can be introduced into host cells via, for example, transformation, transfection, infection, electroporation and sonoporation, so that many copies of the gene sequence are generated.
- 20 In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated SP protein gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene can be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when
- 25 necessary, retrieving the inserted gene from the isolated recombinant DNA.
- E. Vectors, plasmids and cells that contain nucleic acids encoding an SP protein or protease domain thereof and expression of SP proteins**

Vectors and cells

- For recombinant expression of one or more of the SP proteins, the nucleic
- 30 acid containing all or a portion of the nucleotide sequence encoding the SP protein can be inserted into an appropriate expression vector, *i.e.*, a vector that contains the necessary elements for the transcription and translation of the

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inserted protein coding sequence. The necessary transcriptional and translational signals also can be supplied by the native promoter for SP genes, and/or their flanking regions.

Also provided are vectors that contain nucleic acid encoding the SPs.

- 5 Cells containing the vectors are also provided. The cells include eukaryotic and prokaryotic cells, and the vectors are any suitable for use therein.

- Prokaryotic and eukaryotic cells, including endothelial cells, containing the vectors are provided. Such cells include bacterial cells, yeast cells, fungal cells, plant cells, insect cells and animal cells. The cells are used to produce an SP
- 10 protein or protease domain thereof by growing the above-described cells under conditions whereby the encoded SP protein or protease domain of the SP protein is expressed by the cell, and recovering the expressed protease domain protein. For purposes herein, the protease domain can be secreted into the medium.

- In one embodiment, the vectors include a sequence of nucleotides that
- 15 encodes a polypeptide that has protease activity and contains all or a portion of only the protease domain, or multiple copies thereof, of an SP protein are provided. Also provided are vectors that comprise a sequence of nucleotides that encodes the protease domain and additional portions of an SP protein up to and including a full length SP protein, as well as multiple copies thereof, are also
- 20 provided. The vectors can selected for expression of the SP protein or protease domain thereof in the cell or such that the SP protein is expressed as a secreted protein. Alternatively, the vectors can include signals necessary for secretion of encoded proteins. When the protease domain is expressed the nucleic acid is linked to nucleic acid encoding a secretion signal, such as the *Saccharomyces*
- 25 *cerevisiae* α mating factor signal sequence or a portion thereof, or the native signal sequence.

- A variety of host-vector systems can be used to express the protein coding sequence. These include but are not limited to mammalian cell systems infected with virus (*e.g.* vaccinia virus, adenovirus, etc.); insect cell systems
- 30 infected with virus (*e.g.* baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and

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specificities. Depending on the host-vector system used, any one of a number of suitable transcription and translation elements can be used.

Any methods known to those of skill in the art for the insertion of DNA fragments into a vector can be used to construct expression vectors containing a
5 chimeric gene containing of appropriate transcriptional/translational control signals and protein coding sequences. These methods can include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequences encoding SP protein, or domains, derivatives, fragments or homologs thereof, can be regulated by a
10 second nucleic acid sequence so that the genes or fragments thereof are expressed in a host transformed with the recombinant DNA molecule(s). For example, expression of the proteins can be controlled by any promoter/enhancer known in the art. In a specific embodiment, the promoter is not native to the genes for SP protein. Promoters which can be used include but are not limited
15 to the SV40 early promoter (Bernoist and Chambon, *Nature* 290:304-310 (1981)), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto *et al.*, *Cell* 22:787-797 (1980)), the herpes thymidine kinase promoter (Wagner *et al.*, *Proc. Natl. Acad. Sci. USA* 78:1441-1445 (1981)), the regulatory sequences of the metallothionein gene (Brinster *et al.*, *Nature* 296:39-
20 42 (1982)); prokaryotic expression vectors such as the β -lactamase promoter (Villa-Kamaroff *et al.*, *Proc. Natl. Acad. Sci. USA* 75:3727-3731 1978)) or the *tac* promoter (DeBoer *et al.*, *Proc. Natl. Acad. Sci. USA* 80:21-25 (1983)); see also "Useful Proteins from Recombinant Bacteria": in *Scientific American* 242:79-94 (1980)); plant expression vectors containing the nopaline synthetase
25 promoter (Herrar-Estrella *et al.*, *Nature* 303:209-213 (1984)) or the cauliflower mosaic virus 35S RNA promoter (Gardner *et al.*, *Nucleic Acids Res.* 9:2871 (1981)), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella *et al.*, *Nature* 310:115-120 (1984)); promoter elements from yeast and other fungi such as the Gal4 promoter, the alcohol
30 dehydrogenase promoter, the phosphoglycerol kinase promoter, the alkaline phosphatase promoter, and the following animal transcriptional control regions that exhibit tissue specificity and have been used in transgenic animals: elastase

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I gene control region which is active in pancreatic acinar cells (Swift et al., *Cell* 38:639-646 (1984); Ornitz et al., *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409 (1986); MacDonald, *Hepatology* 7:425-515 (1987)); insulin gene control region which is active in pancreatic beta cells (Hanahan et al., *Nature* 5 315:115-122 (1985)), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., *Cell* 38:647-658 (1984); Adams et al., *Nature* 318:533-538 (1985); Alexander et al., *Mol. Cell Biol.* 7:1436-1444 (1987)), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., *Cell* 45:485-495 (1986)), albumin gene control region which is active in liver (Pinckert et al., *Genes and Devel.* 1:268-276 (1987)), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., *Mol. Cell Biol.* 5:1639-1648 (1985); Hammer et al., *Science* 235:53-58 (1987)), alpha-1 antitrypsin gene control region which is active in liver (Kelsey et al., *Genes and Devel.* 1:161-171 (1987)), beta globin gene control region which is active in myeloid cells (Mogram et al., *Nature* 315:338-340 15 (1985); Kollias et al., *Cell* 46:89-94 (1986)), myelin basic protein gene control region which is active in oligodendrocyte cells of the brain (Readhead et al., *Cell* 48:703-712 (1987)), myosin light chain-2 gene control region which is active in skeletal muscle (Sani, *Nature* 314:283-286 (1985)), and gonadotrophic releasing hormone gene control region which is active in gonadotrophs of the 20 hypothalamus (Mason et al., *Science* 234:1372-1378 (1986)).

In a specific embodiment, a vector is used that contains a promoter operably linked to nucleic acids encoding an SP protein, or a domain, fragment, derivative or homolog, thereof, one or more origins of replication, and optionally, 25 one or more selectable markers (e.g., an antibiotic resistance gene). Expression vectors containing the coding sequences, or portions thereof, of an SP protein, is made, for example, by subcloning the coding portions into the EcoRI restriction site of each of the three pGEX vectors (glutathione S-transferase expression vectors (Smith and Johnson, *Gene* 7:31-40 (1988)). This allows for the 30 expression of products in the correct reading frame. Vectors and systems for expression of the protease domains of the SP proteins include the well known *Pichia* vectors (available, for example, from Invitrogen, San Diego, CA),

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particularly those designed for secretion of the encoded proteins. One exemplary vector is described in the EXAMPLES.

Plasmids for transformation of *E. coli* cells, include, for example, the pET expression vectors (see, U.S. patent 4,952,496; available from NOVAGEN, Madison, WI; see, also literature published by Novagen describing the system). Such plasmids include pET 11a, which contains the T7lac promoter, T7 terminator, the inducible *E. coli* lac operator, and the lac repressor gene; pET 12a-c, which contains the T7 promoter, T7 terminator, and the *E. coli* ompT secretion signal; and pET 15b and pET19b (NOVAGEN, Madison, WI), which contain a His-Tag™ leader sequence for use in purification with a His column and a thrombin cleavage site that permits cleavage following purification over the column; the T7-lac promoter region and the T7 terminator.

The vectors are introduced into host cells, such as *Pichia* cells and bacterial cells, such as *E. coli*, and the proteins expressed therein. *Pichia* strains, which are known and readily available, include, for example, GS115. Bacterial hosts can contain chromosomal copies of DNA encoding T7 RNA polymerase operably linked to an inducible promoter, such as the lacUV promoter (see, U.S. Patent No. 4,952,496). Such hosts include, but are not limited to, the lysogenic *E. coli* strain BL21(DE3).

20 Expression and production of proteins

The SP domains, derivatives and analogs can be produced by various methods known in the art. For example, once a recombinant cell expressing an SP protein, or a domain, fragment or derivative thereof, is identified, the individual gene product can be isolated and analyzed. This is achieved by assays based on the physical and/or functional properties of the protein, including, but not limited to, radioactive labeling of the product followed by analysis by gel electrophoresis, immunoassay, cross-linking to marker-labeled product.

The CVSP14 polypeptides can be isolated and purified by standard methods known in the art (either from natural sources or recombinant host cells expressing the complexes or proteins), including but not restricted to column chromatography (e.g., ion exchange, affinity, gel exclusion, reversed-phase high

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pressure, fast protein liquid, etc.), differential centrifugation, differential solubility, or by any other standard technique used for the purification of proteins. Functional properties can be evaluated using any suitable assay known in the art.

5 Alternatively, once an SP protein or its domain or derivative is identified, the amino acid sequence of the protein can be deduced from the nucleotide sequence of the gene which encodes it. As a result, the protein or its domain or derivative can be synthesized by standard chemical methods known in the art (e.g. see Hunkapiller et al, *Nature* 310:105-111 (1984)).

10 Manipulations of SP protein sequences can be made at the protein level. Also contemplated herein are SP protein proteins, domains thereof, derivatives or analogs or fragments thereof, which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage
15 to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications can be carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, etc.

20 In addition, domains, analogs and derivatives of an SP protein can be chemically synthesized. For example, a peptide corresponding to a portion of an SP protein, which includes the desired domain or which mediates the desired activity *in vitro* can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs
25 can be introduced as a substitution or addition into the SP protein sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-aminobutyric acid, ϵ -Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline,
30 hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β -methyl amino acids, Ca-methyl amino acids, Na-methyl amino

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acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

In cases where natural products are suspected of having a mutation or are isolated from new species, the amino acid sequence of the SP protein
5 isolated from the natural source, as well as those expressed *in vitro*, or from synthesized expression vectors *in vivo* or *in vitro*, can be determined from analysis of the DNA sequence, or alternatively, by direct sequencing of the isolated protein. Such analysis can be performed by manual sequencing or through use of an automated amino acid sequenator.

10 In particular, for expression of the protease domain of the CVSP14, it was found to be advantageous to express the protein intracellularly without a signal sequence, which results in accumulation or formation of inclusion bodies containing protease domain. The inclusion bodies are isolated, denatured, solubilized and refolded protease domain, which is then activated by cleavage at
15 the RI site (see, *e.g.*, EXAMPLES).

Modifications

A variety of modification of the SP proteins and domains are contemplated herein. An SP-encoding nucleic acid molecule be modified by any of numerous strategies known in the art (Sambrook *et al.* (1989) *Molecular*
20 *Cloning, A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The sequences can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated *in vitro*. In the production of the gene encoding a domain, derivative or analog of SP, care should be taken to ensure that the
25 modified gene retains the original translational reading frame, uninterrupted by translational stop signals, in the gene region where the desired activity is encoded.

Additionally, the SP-encoding nucleic acid molecules can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination
30 sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy pre-existing ones, to facilitate further *in vitro* modification. Also, as described herein muteins with primary sequence

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alterations, such as replacements of Cys residues and elimination of glycosylation sites are contemplated. Such mutations can be effected by any technique for mutagenesis known in the art, including, but not limited to, chemical mutagenesis and *in vitro* site-directed mutagenesis (Hutchinson et al., *J. Biol. Chem.* 253:6551-6558 (1978)), use of TAB® linkers (Pharmacia). In one embodiment, for example, an SP protein or domain thereof is modified to include a fluorescent label. In other specific embodiments, the SP protein is modified to have a heterofunctional reagent, such heterofunctional reagents can be used to crosslink the members of the complex.

10 The SP proteins can be isolated and purified by standard methods known in the art (either from natural sources or recombinant host cells expressing the complexes or proteins), including but not restricted to column chromatography (*e.g.*, ion exchange, affinity, gel exclusion, reversed-phase high pressure, fast protein liquid, etc.), differential centrifugation, differential solubility, or by any
15 other standard technique used for the purification of proteins. Functional properties can be evaluated using any suitable assay known in the art.

F. Screening methods

 The single chain protease domains, as shown herein, can be used in a variety of methods to identify compounds that modulate the activity thereof. For
20 SPs that exhibit higher activity or expression in tumor cells, compounds that inhibit the proteolytic activity are of particular interest. For any SPs that are active at lower levels in tumor cells, compounds or agents that enhance the activity are potentially of interest. In all instances the identified compounds include agents that are candidate cancer treatments.

25 Several types of assays are exemplified and described herein. It is understood that the protease domains can be used in other assays. It is shown here, however, that the single chain protease domains exhibit catalytic activity. As such they are ideal for *in vitro* screening assays. They can also be used in binding assays.

30 The CVSP14 full length zymogens, activated enzymes, single and two chain protease domains are contemplated for use in any screening assay known to those of skill in the art, including those provided herein. Hence the following

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description, if directed to proteolytic assays is intended to apply to use of a single chain protease domain or a catalytically active portion thereof of any SP, including a CVSP14. Other assays, such as binding assays are provided herein, particularly for use with a CVSP14, including any variants, such as splice
5 variants thereof.

1. Catalytic Assays for identification of agents that modulate the protease activity of an SP protein

Methods for identifying a modulator of the catalytic activity of an SP, particularly a single chain protease domain or catalytically active portion thereof,
10 are provided herein. The methods can be practiced by: a) contacting the CVSP14, a full-length zymogen or activated form, and particularly a single-chain domain thereof, with a substrate of the CVSP14 in the presence of a test substance, and detecting the proteolysis of the substrate, whereby the activity of the CVSP14 is assessed, and comparing the activity to a control. For
15 example, the control can be the activity of the CVSP14 assessed by contacting a CVSP14, including a full-length zymogen or activated form, and particularly a single-chain domain thereof, particularly a single-chain domain thereof, with a substrate of the CVSP14, and detecting the proteolysis of the substrate, whereby the activity of the CVSP14 is assessed. The results in the presence
20 and absence of the test compounds are compared. A difference in the activity indicates that the test substance modulates the activity of the CVSP14. Activators of activation are also contemplated; such assays are discussed below.

In one embodiment a plurality of the test substances are screened simultaneously in the above screening method. In another embodiment, the
25 CVSP14 is isolated from a target cell as a means for then identifying agents that are potentially specific for the target cell.

In another embodiment, a test substance is a therapeutic compound, and whereby a difference of the CVSP14 activity measured in the presence and in the absence of the test substance indicates that the target cell responds to the
30 therapeutic compound.

One method includes the steps of (a) contacting the CVSP14 polypeptide or protease domain thereof with one or a plurality of test compounds under

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conditions conducive to interaction between the ligand and the compounds; and (b) identifying one or more compounds in the plurality that specifically binds to the ligand.

Another method provided herein includes the steps of a) contacting a
5 CVSP14 polypeptide or protease domain thereof with a substrate of the CVSP14 polypeptide, and detecting the proteolysis of the substrate, whereby the activity of the CVSP14 polypeptide is assessed; b) contacting the CVSP14 polypeptide with a substrate of the CVSP14 polypeptide in the presence of a test substance, and detecting the proteolysis of the substrate, whereby the activity of the
10 CVSP14 polypeptide is assessed; and c) comparing the activity of the CVSP14 polypeptide assessed in steps a) and b), whereby the activity measured in step a) differs from the activity measured in step b) indicates that the test substance modulates the activity of the CVSP14 polypeptide.

In another embodiment, a plurality of the test substances are screened
15 simultaneously. In comparing the activity of a CVSP14 polypeptide in the presence and absence of a test substance to assess whether the test substance is a modulator of the CVSP14 polypeptide, it is unnecessary to assay the activity in parallel, although such parallel measurement is typical. It is possible to measure the activity of the CVSP14 polypeptide at one time point and compare
20 the measured activity to a historical value of the activity of the CVSP14 polypeptide.

For instance, one can measure the activity of the CVSP14 polypeptide in the presence of a test substance and compare with historical value of the activity of the CVSP14 polypeptide measured previously in the absence of the
25 test substance, and *vice versa*. This can be accomplished, for example, by providing the activity of the CVSP14 polypeptide on an insert or pamphlet provided with a kit for conducting the assay.

Methods for selecting substrates for a particular SP are described in the EXAMPLES, and particular proteolytic assays are exemplified.

30 Combinations and kits containing the combinations optionally including instructions for performing the assays are provided. The combinations include a CVSP14 polypeptide and a substrate of the CVSP14 polypeptide to be assayed;

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and, optionally reagents for detecting proteolysis of the substrate. The substrates, which can be chromogenic or fluorogenic molecules, including proteins, subject to proteolysis by a particular CVSP14 polypeptide, can be identified empirically by testing the ability of the CVSP14 polypeptide to cleave the test substrate. Substrates that are cleaved most effectively (i.e., at the lowest concentrations and/or fastest rate or under desirable conditions), are identified.

Additionally provided herein is a kit containing the above-described combination. The kit optionally includes instructions for identifying a modulator of the activity of a CVSP14 polypeptide. Any CVSP14 polypeptide is contemplated as target for identifying modulators of the activity thereof.

2. Binding assays

Also provided herein are methods for identification and isolation of agents, particularly compounds that bind to CVSP14s. The assays are designed to identify agents that bind to the zymogen form, the single chain isolated protease domain (or a protein, other than a CVSP14 polypeptide, that contains the protease domain of a CVSP14 polypeptide), and to the activated form, including the activated form derived from the full length zymogen or from an extended protease domain. The identified compounds are candidates or leads for identification of compounds for treatments of tumors and other disorders and diseases involving aberrant angiogenesis. The CVSP14 polypeptides used in the methods include any CVSP14 polypeptide as defined herein, including the CVSP14 single chain protease domain or proteolytically active portion thereof.

A variety of methods are provided herein. These methods can be performed in solution or in solid phase reactions in which the CVSP14 polypeptide(s) or protease domain(s) thereof are linked, either directly or indirectly via a linker, to a solid support. Screening assays are described in the Examples, and these assays have been used to identify candidate compounds. For purposes herein, all binding assays described above are provided for CVSP14.

Methods for identifying an agent, such as a compound, that specifically binds to a CVSP14 single chain protease domain, a zymogen or full-length

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activated CVSP14 or two chain protease domain thereof are provided herein. The method can be practiced by (a) contacting the CVSP14 with one or a plurality of test agents under conditions conducive to binding between the CVSP14 and an agent; and (b) identifying one or more agents within the plurality
5 that specifically binds to the CVSP14.

For example, in practicing such methods the CVSP14 polypeptide is mixed with a potential binding partner or an extract or fraction of a cell under conditions that allow the association of potential binding partners with the polypeptide. After mixing, peptides, polypeptides, proteins or other molecules
10 that have become associated with a CVSP14 are separated from the mixture. The binding partner that bound to the CVSP14 can then be removed and further analyzed. To identify and isolate a binding partner, the entire protein, for instance the entire disclosed protein of SEQ ID Nos. 6 can be used. Alternatively, a fragment of the protein can be used.

15 A variety of methods can be used to obtain cell extracts or body fluids, such as blood, serum, urine, sweat, synovial fluid, CSF and other such fluids. For example, cells can be disrupted using either physical or chemical disruption methods. Examples of physical disruption methods include, but are not limited to, sonication and mechanical shearing. Examples of chemical lysis methods
20 include, but are not limited to, detergent lysis and enzyme lysis. A skilled artisan can readily adapt methods for preparing cellular extracts in order to obtain extracts for use in the present methods.

Once an extract of a cell is prepared, the extract is mixed with the CVSP14 under conditions in which association of the protein with the binding
25 partner can occur. A variety of conditions can be used, including conditions that resemble conditions found in the cytoplasm of a human cell. Features such as osmolarity, pH, temperature, and the concentration of cellular extract used, can be varied to optimize the association of the protein with the binding partner. Similarly, methods for isolation of molecules of interest from body fluids are
30 known.

After mixing under appropriate conditions, the bound complex is separated from the mixture. A variety of techniques can be used to separate the

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mixture. For example, antibodies specific to a CVSP14 can be used to immunoprecipitate the binding partner complex. Alternatively, standard chemical separation techniques such as chromatography and density/sediment centrifugation can be used.

5 After removing the non-associated cellular constituents in the extract, the binding partner can be dissociated from the complex using conventional methods. For example, dissociation can be accomplished by altering the salt concentration or pH of the mixture.

10 To aid in separating associated binding partner pairs from the mixed extract, the CVSP14 can be immobilized on a solid support. For example, the protein can be attached to a nitrocellulose matrix or acrylic beads. Attachment of the protein or a fragment thereof to a solid support aids in separating peptide/binding partner pairs from other constituents found in the extract. The identified binding partners can be either a single protein or a complex made up of
15 two or more proteins.

 Alternatively, the nucleic acid molecules encoding the single chain proteases can be used in a yeast two-hybrid system. The yeast two-hybrid system has been used to identify other protein partner pairs and can readily be adapted to employ the nucleic acid molecules herein described.

20 Another *in vitro* binding assay, particularly for a CVSP14, uses a mixture of a polypeptide that contains at least the catalytic domain of one of these proteins and one or more candidate binding targets or substrates. After incubating the mixture under appropriate conditions, the ability of the CVSP14 or a polypeptide fragment thereof containing the catalytic domain to bind to or
25 interact with the candidate substrate is assessed. For cell-free binding assays, one of the components includes or is coupled to a detectable label. The label can provide for direct detection, such as radioactivity, luminescence, optical or electron density, *etc.*, or indirect detection such as an epitope tag, an enzyme, *etc.* A variety of methods can be employed to detect the label depending on the
30 nature of the label and other assay components. For example, the label can be detected bound to the solid substrate or a portion of the bound complex

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containing the label can be separated from the solid substrate, and the label thereafter detected.

3. Detection of signal transduction

Secreted CVSPs, such as CVSP14, can be involved in signal transduction either directly by binding to or interacting with a cell surface receptor or indirectly by activating proteins, such as pro-growth factors that can initiate signal transduction. Assays for assessing signal transduction are well known to those of skill in the art, and can be adapted for use with the CVSP14 polypeptide.

Assays for identifying agents that affect or alter signal transduction mediated directly or indirectly, such as via activation of a pro-growth factor, by a CVSP14, particularly the full length or a sufficient portion to anchor the extracellular domain or a functional portion thereof of a CVSP on the surface of a cell are provided. Such assays, include, for example, transcription based assays in which modulation of a transduced signal is assessed by detecting an effect on an expression from a reporter gene (see, *e.g.*, U.S. Patent No. 5,436,128).

4. Methods for Identifying Agents that Modulate the Expression a Nucleic Acid Encoding a CVSP14

Another embodiment provides methods for identifying agents that modulate the expression of a nucleic acid encoding a CVSP14. Such assays use any available means of monitoring for changes in the expression level of the nucleic acids encoding a CVSP14.

In one assay format, cell lines that contain reporter gene fusions between the open reading frame of CVSP14 or a domain thereof, particularly the protease domain and any assayable fusion partner can be prepared. Numerous assayable fusion partners are known and readily available including the firefly luciferase gene and the gene encoding chloramphenicol acetyltransferase (Alam *et al.*, *Anal. Biochem.* 188: 245-54 (1990)). Cell lines containing the reporter gene fusions are then exposed to the agent to be tested under appropriate conditions and time. Differential expression of the reporter gene between samples exposed to the agent and control samples identifies agents which modulate the expression of a nucleic acid encoding a CVSP14.

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Additional assay formats can be used to monitor the ability of the agent to modulate the expression of a nucleic acid encoding a CVSP14. For instance, mRNA expression can be monitored directly by hybridization to the nucleic acids. Cell lines are exposed to the agent to be tested under appropriate conditions and time and total RNA or mRNA is isolated by standard procedures (see, *e.g.*,
5 Sambrook *et al.* (1989) MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed. Cold Spring Harbor Laboratory Press). Probes to detect differences in RNA expression levels between cells exposed to the agent and control cells can be prepared from the nucleic acids. It is typical, but not necessary, to design
10 probes which hybridize only with target nucleic acids under conditions of high stringency. Only highly complementary nucleic acid hybrids form under conditions of high stringency. Accordingly, the stringency of the assay conditions determines the amount of complementarity which should exist between two nucleic acid strands in order to form a hybrid. Stringency should
15 be chosen to maximize the difference in stability between the probe:target hybrid and potential probe:non-target hybrids.

Probes can be designed from the nucleic acids through methods known in the art. For instance, the G + C content of the probe and the probe length can affect probe binding to its target sequence. Methods to optimize probe
20 specificity are commonly available (see, *e.g.*, Sambrook *et al.* (1989) MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed. Cold Spring Harbor Laboratory Press); and Ausubel *et al.* (1995) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing Co., NY).

Hybridization conditions are modified using known methods (see, *e.g.*,
25 Sambrook *et al.* (1989) MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed. Cold Spring Harbor Laboratory Press); and Ausubel *et al.* (1995) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing Co., NY), as required for each probe. Hybridization of total cellular RNA or RNA enriched for polyA RNA can be accomplished in any available format. For instance, total
30 cellular RNA or RNA enriched for polyA RNA can be affixed to a solid support, and the solid support exposed to at least one probe comprising at least one, or part of one of the nucleic acid molecules under conditions in which the probe

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specifically hybridizes. Alternatively, nucleic acid fragments comprising at least one, or part of one of the sequences can be affixed to a solid support, such as a porous glass wafer. The glass wafer can then be exposed to total cellular RNA or polyA RNA from a sample under conditions in which the affixed sequences

5 specifically hybridize. Such glass wafers and hybridization methods are widely available, for example, those disclosed by Beattie (WO 95/11755). By examining for the ability of a given probe to specifically hybridize to an RNA sample from an untreated cell population and from a cell population exposed to the agent, agents which up or down regulate the expression of a nucleic acid

10 encoding the CVSP14 polypeptide, are identified.

In one format, the relative amounts of a protein between a cell population that has been exposed to the agent to be tested compared to an un-exposed control cell population can be assayed (*e.g.*, a prostate cancer cell line, a lung cancer cell line, a colon cancer cell line or a breast cancer cell line). In this

15 format, probes, such as specific antibodies, are used to monitor the differential expression or level of activity of the protein in the different cell populations or body fluids. Cell lines or populations or body fluids are exposed to the agent to be tested under appropriate conditions and time. Cellular lysates or body fluids can be prepared from the exposed cell line or population and a control,

20 unexposed cell line or population or unexposed body fluid. The cellular lysates or body fluids are then analyzed with the probe.

For example, N- and C- terminal fragments of the CVSP14 can be expressed in bacteria and used to search for proteins which bind to these fragments. Fusion proteins, such as His-tag or GST fusion to the N- or C-

25 terminal regions of the CVSP14 can be prepared for use as a substrate. These fusion proteins can be coupled to, for example, Glutathione-Sepharose beads and then probed with cell lysates or body fluids. Prior to lysis, the cells or body fluids can be treated with a candidate agent which can modulate a CVSP14 or proteins that interact with domains thereon. Lysate proteins binding to the

30 fusion proteins can be resolved by SDS-PAGE, isolated and identified by protein sequencing or mass spectroscopy, as is known in the art.

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Antibody probes are prepared by immunizing suitable mammalian hosts in appropriate immunization protocols using the peptides, polypeptides or proteins if they are of sufficient length (*e.g.*, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40 or more consecutive amino acids the CVSP14 polypeptide or if
5 required to enhance immunogenicity, conjugated to suitable carriers. Methods for preparing immunogenic conjugates with carriers, such as bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH), or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents can be effective; in other instances linking
10 reagents such as those supplied by Pierce Chemical Co., Rockford, IL, can be desirable to provide accessibility to the hapten. Hapten peptides can be extended at either the amino or carboxy terminus with a Cys residue or interspersed with cysteine residues, for example, to facilitate linking to a carrier. Administration of the immunogens is conducted generally by injection over a
15 suitable time period and with use of suitable adjuvants, as is generally understood in the art. During the immunization schedule, titers of antibodies are taken to determine adequacy of antibody formation.

Anti-peptide antibodies can be generated using synthetic peptides corresponding to, for example, the carboxy terminal amino acids of the CVSP14.
20 Synthetic peptides can be as small as 1-3 amino acids in length, generally at least 4 or more amino acid residues long. The peptides can be coupled to KLH using standard methods and can be immunized into animals, such as rabbits or ungulate. Polyclonal antibodies can then be purified, for example using Actigel beads containing the covalently bound peptide.

25 While the polyclonal antisera produced in this way can be satisfactory for some applications, for pharmaceutical compositions, use of monoclonal preparations are generally used. Immortalized cell lines which secrete the desired monoclonal antibodies can be prepared using the standard method of Kohler *et al.*, (*Nature* 256: 495-7 (1975)) or modifications which effect
30 immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the peptide hapten, polypeptide or protein.

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When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either *in vitro* or by production *in vivo* via ascites fluid. Of particular interest, are monoclonal antibodies that recognize the catalytic domain of the α CVSP14.

5 Additionally, the zymogen or two-chain form of the CVSP14 can be used to make monoclonal antibodies that recognize conformation epitopes. The desired monoclonal antibodies are then recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can be used as
10 antagonists, as well as the intact antibodies. Use of immunologically reactive fragments, such as the Fab, Fab', of F(ab')₂ fragments are often used, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole immunoglobulin.

 The antibodies or fragments can also be produced. Regions that bind
15 specifically to the desired regions of receptor also can be produced in the context of chimeras with multiple species origin.

 Agents that are assayed in the above method can be randomly selected or rationally selected or designed.

 The agents can be, as examples, peptides, small molecules, and
20 carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents.

 The peptide agents can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides can be synthesized using commercially
25 available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

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G. Assay formats and selection of test substances that modulate at least one activity of a CVSP14 polypeptide

Methods for identifying agents that modulate at least one activity of a CVSP14 are provided. The methods include phage display and other methods for assessing alterations in the activity of a CVSP14. Such methods or assays can use any means of monitoring or detecting the desired activity. A variety of formats and detection protocols are known for performing screening assays. Any such formats and protocols can be adapted for identifying modulators of CVSP14 polypeptide activities. The following includes a discussion of exemplary protocols.

1. High throughput screening assays

Although the above-described assay can be conducted where a single CVSP14 polypeptide is screened, and/or a single test substance is screened in one assay, the assay typically is conducted in a high throughput screening mode, *i.e.*, a plurality of the SP proteins are screened against and/or a plurality of the test substances are screened simultaneously (*See generally, High Throughput Screening: The Discovery of Bioactive Substances* (Devlin, Ed.) Marcel Dekker, 1997; Sittampalam et al., *Curr. Opin. Chem. Biol.*, 1:384-91 (1997); and Silverman et al., *Curr. Opin. Chem. Biol.*, 2:397-403 (1998)). For example, the assay can be conducted in a multi-well (*e.g.*, 24-, 48-, 96-, 384-, 1536-well or higher density), chip or array format.

High-throughput screening (HTS) is the process of testing a large number of diverse chemical structures against disease targets to identify "hits" (Sittampalam et al., *Curr. Opin. Chem. Biol.*, 1:384-91 (1997)). Current state-of-the-art HTS operations are highly automated and computerized to handle sample preparation, assay procedures and the subsequent processing of large volumes of data.

Detection technologies employed in high-throughput screens depend on the type of biochemical pathway being investigated (Sittampalam et al., *Curr. Opin. Chem. Biol.*, 1:384-91 (1997)). These methods include, radiochemical methods, such as the scintillation proximity assays (SPA), which can be adapted to a variety of enzyme assays (Lerner et al., *J. Biomol. Screening*, 1:135-143

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(1996); Baker et al., *Anal. Biochem.*, 239:20-24 (1996); Baum et al., *Anal. Biochem.*, 237:129-134 (1996); and Sullivan et al., *J. Biomol. Screening* 2:19-23 (1997)) and protein-protein interaction assays (Braunwalder et al., *J. Biomol. Screening* 1:23-26 (1996); Sonatore et al., *Anal. Biochem.* 240:289-297 (1996); and Chen et al., *J. Biol. Chem.* 271:25308-25315 (1996)), and non-isotopic detection methods, including but are not limited to, colorimetric and luminescence detection methods, resonance energy transfer (RET) methods, time-resolved fluorescence (HTRF) methods, cell-based fluorescence assays, such as fluorescence resonance energy transfer (FRET) procedures (see, e.g., Gonzalez et al., *Biophys. J.*, 69:1272-1280 (1995)), fluorescence polarization or anisotropy methods (see, e.g., Jameson et al., *Methods Enzymol.* 246:283-300 (1995); Jolley, *J. Biomol. Screening* 1:33-38 (1996); Lynch et al., *Anal. Biochem.* 247:77-82 (1997)), fluorescence correlation spectroscopy (FCS) and other such methods.

2. Test Substances

Test compounds, including small molecules, antibodies, proteins, nucleic acids, peptides, and libraries and collections thereof, can be screened in the above-described assays and assays described below to identify compounds that modulate the activity of a CVSP14 polypeptide. Rational drug design methodologies that rely on computational chemistry can be used to screen and identify candidate compounds.

The compounds identified by the screening methods include inhibitors, including antagonists, and can be agonists. Compounds for screening include any compounds and collections of compounds available, known or that can be prepared.

a. Selection of Compounds

Compounds can be selected for their potency and selectivity of inhibition of serine proteases, especially a CVSP14 polypeptide. As described herein, and as generally known, a target serine protease and its substrate are combined under assay conditions permitting reaction of the protease with its substrate. The assay is performed in the absence of test compound, and in the presence of increasing concentrations of the test compound. The concentration of test

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compound at which 50% of the serine protease activity is inhibited by the test compound is the IC_{50} value (Inhibitory Concentration) or EC_{50} (Effective Concentration) value for that compound. Within a series or group of test compounds, those having lower IC_{50} or EC_{50} values are considered more potent inhibitors of the serine protease than those compounds having higher IC_{50} or EC_{50} values. The IC_{50} measurement is often used for more simplistic assays, whereas the EC_{50} is often used for more complicated assays, such as those employing cells.

Typically candidate compounds have an IC_{50} value of 100 nM or less as measured in an *in vitro* assay for inhibition of CVSP14 polypeptide activity. The test compounds also are evaluated for selectivity toward a serine protease. As described herein, and as generally known, a test compound is assayed for its potency toward a panel of serine proteases and other enzymes and an IC_{50} value or EC_{50} value is determined for each test compound in each assay system. A compound that demonstrates a low IC_{50} value or EC_{50} value for the target enzyme, *e.g.*, CVSP14 polypeptide, and a higher IC_{50} value or EC_{50} value for other enzymes within the test panel (*e.g.*, urokinase tissue plasminogen activator, thrombin, Factor Xa), is considered to be selective toward the target enzyme. Generally, a compound is deemed selective if its IC_{50} value or EC_{50} value in the target enzyme assay is at least one order of magnitude less than the next smallest IC_{50} value or EC_{50} value measured in the selectivity panel of enzymes.

Compounds are also evaluated for their activity *in vivo*. The type of assay chosen for evaluation of test compounds depends on the pathological condition to be treated or prevented by use of the compound, as well as the route of administration to be evaluated for the test compound.

For instance, to evaluate the activity of a compound to reduce tumor growth through inhibition of CVSP14 polypeptide, the procedures described by Jankun et al., *Canc. Res.* 57:559-563 (1997) to evaluate PAI-1 can be employed. Briefly, the ATCC cell lines DU145 and LnCaP are injected into SCID mice. After tumors are established, the mice are given test compound according to a dosing regime determined from the compound's *in vitro* characteristics. The

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Jankun *et al.* compound was administered in water. Tumor volume measurements are taken twice a week for about five weeks. A compound is deemed active if an animal to which the compound was administered exhibited decreased tumor volume, as compared to animals receiving appropriate control compounds.

Another *in vivo* experimental model designed to evaluate the effect of p-aminobenzamidine, a swine protease inhibitor, on reducing tumor volume is described by Billström *et al.*, *Int. J. Cancer* 61:542-547 (1995).

To evaluate the ability of a compound to reduce the occurrence of, or inhibit, metastasis, the procedures described by Kobayashi *et al.* *Int. J. Canc.* 57:727-733d (1994) can be employed. Briefly, a murine xenograft selected for high lung colonization potential is injected into C57B1/6 mice i.v. (experimental metastasis) or s.c. into the abdominal wall (spontaneous metastasis). Various concentrations of the compound to be tested can be admixed with the tumor cells in Matrigel prior to injection. Daily i.p. injections of the test compound are made either on days 1-6 or days 7-13 after tumor inoculation. The animals are sacrificed about three or four weeks after tumor inoculation, and the lung tumor colonies are counted. Evaluation of the resulting data permits a determination as to efficacy of the test compound, optimal dosing and route of administration.

The activity of the tested compounds toward decreasing tumor volume and metastasis can be evaluated in model described in Rabbani *et al.*, *Int. J. Cancer* 63:840-845 (1995) to evaluate their inhibitor. There, Mat LyLu tumor cells were injected into the flank of Copenhagen rats. The animals were implanted with osmotic minipumps to continuously administer various doses of test compound for up to three weeks. The tumor mass and volume of experimental and control animals were evaluated during the experiment, as were metastatic growths. Evaluation of the resulting data permits a determination as to efficacy of the test compound, optimal dosing, and route of administration. Some of these authors described a related protocol in Xing *et al.*, *Canc. Res.* 57:3585-3593 (1997).

To evaluate the anti-angiogenesis activity of a compound, a rabbit cornea neovascularization model can be employed (see, *e.g.*, Avery *et al.* (1990) *Arch.*

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Ophthalmol., 108:1474-147). Avery *et al.* describes anesthetizing New Zealand albino rabbits and then making a central corneal incision and forming a radial corneal pocket. A slow release prostaglandin pellet was placed in the pocket to induce neovascularization. Test compound was administered i.p. for five days, at which time the animals were sacrificed. The effect of the test compound is evaluated by review of periodic photographs taken of the limbus, which can be used to calculate the area of neovascular response and, therefore, limbal neovascularization. A decreased area of neovascularization as compared with appropriate controls indicates the test compound was effective at decreasing or inhibiting neovascularization.

An angiogenesis model used to evaluate the effect of a test compound in preventing angiogenesis is described by Min *et al. Canc. Res.* 56:2428-2433 (1996). C57BL6 mice receive subcutaneous injections of a Matrigel mixture containing bFGF, as the angiogenesis-inducing agent, with and without the test compound. After five days, the animals are sacrificed and the Matrigel plugs, in which neovascularization can be visualized, are photographed. An experimental animal receiving Matrigel and an effective dose of test compound exhibits less vascularization than a control animal or an experimental animal receiving a less- or non-effective dose of compound.

An *in vivo* system designed to test compounds for their ability to limit the spread of primary tumors is described by Crowley *et al., Proc. Natl. Acad. Sci.* 90:5021-5025 (1993). Nude mice are injected with tumor cells (PC3) engineered to express CAT (chloramphenicol acetyltransferase). Compounds to be tested for their ability to decrease tumor size and/or metastases are administered to the animals, and subsequent measurements of tumor size and/or metastatic growths are made. In addition, the level of CAT detected in various organs provides an indication of the ability of the test compound to inhibit metastasis; detection of less CAT in tissues of a treated animal versus a control animal indicates less CAT-expressing cells migrated to that tissue.

In vivo experimental modes designed to evaluate the inhibitory potential of a test serine protease inhibitors, using a tumor cell line F3ll known to be highly invasive (see, *e.g.*, Alonso *et al., Breast Canc. Res. Treat.* 40:209-223

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(1996)). Alonso describes *in vivo* studies for toxicity determination, tumor growth, invasiveness, spontaneous metastasis, experimental lung metastasis, and an angiogenesis assay.

The CAM model (chick embryo chorioallantoic membrane model), first
5 described by L. Ossowski in 1998 (*J. Cell Biol.* 107:2437-2445 (1988)),
provides another method for evaluating the inhibitory activity of a test
compound. In the CAM model, tumor cells invade through the chorioallantoic
membrane containing CAM with tumor cells in the presence of several serine
protease inhibitors results in less or no invasion of the tumor cells through the
10 membrane. Thus, the CAM assay is performed with CAM and tumor cells in the
presence and absence of various concentrations of test compound. The
invasiveness of tumor cells is measured under such conditions to provide an
indication of the compound's inhibitory activity. A compound having inhibitory
activity correlates with less tumor invasion.

15 The CAM model is also used in a standard assay of angiogenesis (*i.e.*,
effect on formation of new blood vessels (Brooks *et al. Methods in Molecular
Biology* 129:257-269 (1999)). According to this model, a filter disc containing
an angiogenesis inducer, such as basic fibroblast growth factor (bFDG) is placed
onto the CAM. Diffusion of the cytokine into the CAM induces local
20 angiogenesis, which can be measured in several ways such as by counting the
number of blood vessel branch points within the CAM directly below the filter
disc. The ability of identified compounds to inhibit cytokine-induced
angiogenesis can be tested using this model. A test compound can either be
added to the filter disc that contains the angiogenesis inducer, be placed directly
25 on the membrane or be administered systemically. The extent of new blood
vessel formation in the presence and/or absence of test compound can be
compared using this model. The formation of fewer new blood vessels in the
presence of a test compound would be indicative of anti-angiogenesis activity.

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Demonstration of anti-angiogenesis activity for inhibitors of a CVSP14 polypeptide indicates a role in angiogenesis for that SP protein.

b. Known serine protease inhibitors

- Compounds for screening can be serine protease inhibitors, which can be
- 5 tested for their ability to inhibit the activity of a CVSP14.
- Exemplary, serine protease inhibitors for use in the screening assays, include, but are not limited to: Serine Protease Inhibitor 3 (SPI-3) (Chen, *et al. Citokine*, 11:856-862 (1999)); Aprotinin (Iijima, R., *et al., J. Biochem. (Tokyo)* 126:912-916 (1999)); Kazal-type serine protease inhibitor-like proteins (Niimi, *et al. Eur.*
- 10 *J. Biochem.*, 266:282-292 (1999)); Kunitz-type serine protease inhibitor (Ravichandran, S., *et al., Acta Crystallogr. D. Biol. Crystallogr.*, 55:1814-1821 (1999)); Tissue factor pathway inhibitor-2/Matrix-associated serine protease inhibitor (TFPI-2/MSPI), (Liu, Y. *et al. Arch. Biochem. Biophys.* 370:112-8 (1999)); Bukunin (Cui, C.Y. *et al. J. Invest. Dermatol.* 113:182-8 (1999));
- 15 Nafmostat mesilate (Ryo, R. *et al. Vox Sang.* 76:241-6 (1999)); TPCK (Huang *et al. Oncogene* 18:3431-3439 (1999)); A synthetic cotton-bound serine protease inhibitor (Edwards *et al. Wound Repair Regen.* 7:106-18 (1999)); FUT-175 (Sawada, M. *et al. Stroke* 30:644-50 (1999)); Combination of serine protease inhibitor FUT-0175 and thromboxane synthetase inhibitor OKY-046
- 20 (Kaminogo *et al. Neurol. Med. Chir. (Tokyo)* 38:704-8; discussion 708-9 (1998)); The rat serine protease inhibitor 2.1 gene (LeCam, A., *et al., Biochem. Biophys. Res. Commun.*, 253:311-4 (1998)); A new intracellular serine protease inhibitor expressed in the rat pituitary gland complexes with granzyme B (Hill *et al. FEBS Lett.* 440:361-4 (1998)); 3,4-Dichloroisocoumarin (Hammed *et al. Proc.*
- 25 *Soc. Exp. Biol. Med.*, 219:132-7 (1998)); LEX032 (Bains *et al. Eur. J. Pharmacol.* 356:67-72 (1998)); N-tosyl-L-phenylalanine chloromethyl ketone (Dryjanski *et al. Biochemistry* 37:14151-6 (1998)); Mouse gene for the serine protease inhibitor neuroserpin (P112) (Berger *et al. Gene*, 214:25-33 (1998)); Rat serine protease inhibitor 2.3 gene (Paul *et al. Eur. J. Biochem.* 254:538-46
- 30 (1998)); Ecotin (Yang *et al. J. Mol. Biol.* 279:945-57 (1998)); A 14 kDa plant-related serine protease inhibitor (Roch *et al. Dev. Comp. Immunol.* 22(1):1-12 (1998)); Matrix-associated serine protease inhibitor TFPI-2/33 kDa MSPI (Rao *et*

- al. Int. J. Cancer* 76:749-56 (1998)); ONO-3403 (Hiwasa *et al. Cancer Lett.* 126:221-5 (1998)); Bdegalastasin (Moser *et al. Eur. J. Biochem.* 253:212-20 (1998)); Bikunin (Xu *et al. J. Mol. Biol.* 276:955-66 (1998)); Nafamostat mesilate (Mellgren *et al. Thromb. Haemost.* 79:342-7 (1998)); The growth
5. hormone dependent serine protease inhibitor, Spi 2.1 (Maake *et al. Endocrinology* 138:5630-6 (1997)); Growth factor activator inhibitor type 2, a Kunitz-type serine protease inhibitor (Kawaguchi *et al. J. Biol. Chem.*, 272:27558-64 (1997)); Heat-stable serine protease inhibitor protein from ovaries of the desert locust, *Schistocerca gregaria* (Hamdaoui *et al. Biochem. Biophys. Res. Commun.* 238:357-60 (1997)); Human placental Hepatocyte growth factor activator inhibitor, a Kunitz-type serine protease inhibitor (Shimomura *et al. J. Biol. Chem.* 272:6370-6 (1997)); FUT-187, oral serine protease inhibitor (Shiozaki *et al. Gan To Kagaku Ryoho*, 23(14): 1971-9 (1996)); Extracellular matrix-associated serine protease inhibitors (Mr 33,000, 31,000, and 27,000
 - 15 (Rao, C.N., *et al., Arch. Biochem. Biophys.*, 335:82-92 (1996)); An irreversible isocoumarin serine protease inhibitor (Palencia, D.D., *et al., Biol. Reprod.*, 55:536-42 (1996)); 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF) (Nakabo *et al. J. Leukoc. Biol.* 60:328-36 (1996)); Neuroserpin (Osterwalder, T., *et al., EMBO J.* 15:2944-53 (1996)); Human serine protease inhibitor alpha-1-
 - 20 antitrypsin (Forney *et al. J. Parasitol.* 82:496-502 (1996)); Rat serine protease inhibitor 2.3 (Simar-Blanchet, A.E., *et al., Eur. J. Biochem.*, 236:638-48 (1996)); Gebaxate mesilate (parodi, F., *et al., J. Cardiothorac. Vasc. Anesth.* 10:235-7 (1996)); Recombinant serine protease inhibitor, CPTI II (Stankiewicz, M., *et al., (Acta Biochim. Pol.*, 43(3):525-9 (1996)); A cysteine-rich serine protease
 - 25 inhibitor (Guamerin II) (Kim, D.R., *et al., J. Enzym. Inhib.*, 10:81-91 (1996)); Diisopropylfluorophosphate (Lundqvist, H., *et al., Inflamm. Res.*, 44(12):510-7 (1995)); Nexin 1 (Yu, D.W., *et al., J. Cell Sci.*, 108(Pt 12):3867-74 (1995)); LEX032 (Scalia, R., *et al., Shock*, 4(4):251-6 (1995)); Protease nexin I (Houenou, L.J., *et al., Proc. Natl. Acad. Sci. U.S.A.*, 92(3):895-9 (1995));
 - 30 Chymase-directed serine protease inhibitor (Woodard S.L., *et al., J. Immunol.*, 153(11):5016-25 (1994)); N-alpha-tosyl-L-lysyl-chloromethyl ketone (TLCK) (Bourinbaier, A.S., *et al., Cell Immunol.*, 155(1):230-6 (1994)); Smpi56

- (Ghendler, Y., et al., *Exp. Parasitol.*, 78(2):121-31 (1994)); Schistosoma haematobium serine protease (Blanton, R.E., et al., *Mol. Biochem. Parasitol.*, 63(1):1-11 (1994)); Spi-1 (Warren, W.C., et al., *Mol. Cell Endocrinol.*, 98(1):27-32 (1993)); TAME (Jessop, J.J., et al., *Inflammation*, 17(5):613-31 (1993));
- 5 Antithrombin III (Kalaria, R.N., et al., *Am. J. Pathol.*, 143(3):886-93 (1993)); FOY-305 (Ohkoshi, M., et al., *Anticancer Res.*, 13(4):963-6 (1993)); Camostat mesilate (Senda, S., et al., *Intern. Med.*, 32(4):350-4 (1993)); Pigment epithelium-derived factor (Steele, F.R., et al., *Proc. Natl. Acad. Sci. U.S.A.*, 90(4):1526-30 (1993)); Antistatin (Holstein, T.W., et al., *FEBS Lett.*, 309(3):288-92 (1992));
- 10 The vaccinia virus K2L gene encodes a serine protease inhibitor (Zhou, J., et al., *Virology*, 189(2):678-86 (1992)); Bowman-Birk serine-protease inhibitor (Werner, M.H., et al., *J. Mol. Biol.*, 225(3):873-89 (1992)); FUT-175 (Yanamoto, H., et al., *Neurosurgery*, 30(3):358-63 (1992)); FUT-175; (Yanamoto, H., et al., *Neurosurgery*, 30(3):351-6, discussion 356-7 (1992));
- 15 PAI-I (Yreadwell, B.V., et al., *J. Orthop. Res.*, 9(3):309-16 (1991)); 3,4-Dichloroisocoumarin (Rusbridge, N.M., et al., *FEBS Lett.*, 268(1):133-6 (1990)); Alpha 1-antichymotrypsin (Lindmark, B.E., et al., *Am. Rev. Respir. Dis.*, 141(4 Pt 1):884-8 (1990)); P-toluenesulfonyl-L-arginine methyl ester (TAME) (Scuderi, P., *J. Immunol.*, 143(1):168-73 (1989)); Alpha 1-antichymotrypsin (Abraham,
- 20 C.R., et al., *Cell*, 52(4):487-501 (1988)); Contrapsin (Modha, J., et al., *Parasitology*, 96 (Pt 1):99-109 (1988)); Alpha 2-antiplasmin (Holmes, W.E., et al., *J. Biol. Chem.*, 262(4):1659-64 (1987)); 3,4-dichloroisocoumarin (Harper, J.W., et al., *Biochemistry*, 24(8):1831-41 (1985)); Diisopropylfluorophosphate (Tsutsui, K., et al., *Biochem. Biophys. Res. Commun.*, 123(1):271-7 (1984));
- 25 Gabexate mesilate (Hesse, B., et al., *Pharmacol. Res. Commun.*, 16(7):637-45 (1984)); Phenyl methyl sulfonyl fluoride (Dufer, J., et al., *Scand. J. Haematol.*, 32(1):25-32 (1984)); Protease inhibitor CI-2 (McPhalen, C.A., et al., *J. Mol. Biol.*, 168(2):445-7 (1983)); Phenylmethylsulfonyl fluoride (Sekar V., et al., *Biochem. Biophys. Res. Commun.*, 89(2):474-8 (1979)); PGE1 (Feinstein, M.D.,
- 30 et al., *Prostaglandine*, 14(6):1075-93 (1977).

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c. Combinatorial libraries and other libraries

The source of compounds for the screening assays, can be libraries, including, but are not limited to, combinatorial libraries. Methods for synthesizing combinatorial libraries and characteristics of such combinatorial
5 libraries are known in the art (*See generally, Combinatorial Libraries: Synthesis, Screening and Application Potential* (Cortese Ed.) Walter de Gruyter, Inc., 1995; Tietze and Lieb, *Curr. Opin. Chem. Biol.*, 2(3):363-71 (1998); Lam, *Anticancer Drug Des.*, 12(3):145-67 (1997); Blaney and Martin, *Curr. Opin. Chem. Biol.*, 1(1):54-9 (1997); and Schultz and Schultz, *Biotechnol. Prog.*, 12(6):729-43
10 (1996)).

Methods and strategies for generating diverse libraries, primarily peptide- and nucleotide-based oligomer libraries, have been developed using molecular biology methods and/or simultaneous chemical synthesis methodologies (*see, e.g.*, Dower et al., *Annu. Rep. Med. Chem.*, 26:271-280 (1991); Fodor et al.,
15 *Science*, 251:767-773 (1991); Jung et al., *Angew. Chem. Int. Ed. Engl.*, 31:367-383 (1992); Zuckerman et al., *Proc. Natl. Acad. Sci. USA*, 89:4505-4509 (1992); Scott et al., *Science*, 249:386-390 (1990); Devlin et al., *Science*, 249:404-406 (1990); Cwirla et al., *Proc. Natl. Acad. Sci. USA*, 87:6378-6382 (1990); and Gallop et al., *J. Medicinal Chemistry*, 37:1233-1251 (1994)). The
20 resulting combinatorial libraries potentially contain millions of compounds and that can be screened to identify compounds that exhibit a selected activity.

The libraries fall into roughly three categories: fusion-protein-displayed peptide libraries in which random peptides or proteins are presented on the surface of phage particles or proteins expressed from plasmids; support-bound
25 synthetic chemical libraries in which individual compounds or mixtures of compounds are presented on insoluble matrices, such as resin beads (*see, e.g.*, Lam et al., *Nature*, 354:82-84 (1991)) and cotton supports (*see, e.g.*, Eichler et al., *Biochemistry* 32:11035-11041 (1993)); and methods in which the compounds are used in solution (*see, e.g.*, Houghten et al., *Nature*, 354:84-86
30 (1991); Houghten et al., *BioTechniques*, 313:412-421 (1992); and Scott et al., *Curr. Opin. Biotechnol.*, 5:40-48 (1994)). There are numerous examples of synthetic peptide and oligonucleotide combinatorial libraries and there are many

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methods for producing libraries that contain non-peptidic small organic molecules. Such libraries can be based on basis set of monomers that are combined to form mixtures of diverse organic molecules or that can be combined to form a library based upon a selected pharmacophore monomer.

- 5 Either a random or a deterministic combinatorial library can be screened by the presently disclosed and/or claimed screening methods. In either of these two libraries, each unit of the library is isolated and/or immobilized on a solid support. In the deterministic library, one knows *a priori* a particular unit's location on each solid support. In a random library, the location of a particular
- 10 unit is not known *a priori* although each site still contains a single unique unit. Many methods for preparing libraries are known to those of skill in this art (see, *e.g.*, Geysen et al., *Proc. Natl. Acad. Sci. USA*, 81:3998-4002 (1984), Houghten et al., *Proc. Natl. Acad. Sci. USA*, 81:5131-5135 (1985)).
- Combinatorial library generated by the any techniques known to those of skill in
- 15 the art are contemplated (see, *e.g.*, Table 1 of Schultz and Schultz, *Biotechnol. Prog.*, 12(6):729-43 (1996)) for screening; Bartel et al., *Science*, 261:1411-1418 (1993); Baumbach et al. *BioPharm*, (Can):24-35 (1992); Bock et al. *Nature*, 355:564-566 (1992); Borman, S., Combinatorial chemists focus on small molecules molecular recognition, and automation, *Chem. Eng. News*,
- 20 2(12):29 (1996); Boublik, et al., Eukaryotic Virus Display: Engineering the Major Surface Glycoproteins of the Autographa California Nuclear Polyhedrosis Virus (ACNPV) for the Presentation of Foreign Proteins on the Virus Surface, *Bio/Technology*, 13:1079-1084 (1995); Brenner, et al., Encoded Combinatorial Chemistry, *Proc. Natl. Acad. Sci. U.S.A.*, 89:5381-5383 (1992); Caffisch, et al.,
- 25 Computational Combinatorial Chemistry for *De Novo* Ligand Design: Review and Assessment, *Perspect. Drug Discovery Des.*, 3:51-84 (1995); Cheng, et al., Sequence-Selective Peptide Binding with a Peptido-A,B-*trans*-steroidal Receptor Selected from an Encoded Combinatorial Library, *J. Am. Chem. Soc.*, 118:1813-1814 (1996); Chu, et al., Affinity Capillary Electrophoresis to Identify the
- 30 Peptide in A Peptide Library that Binds Most Tightly to Vancomycin, *J. Org. Chem.*, 58:648-652 (1993); Clackson, et al., Making Antibody Fragments Using Phage Display Libraries, *Nature*, 352:624-628 (1991); Combs, et al., Protein

- Structure-Based Combinatorial Chemistry: Discovery of Non-Peptide Binding Elements to Src SH3 Domain, *J. Am. Chem. Soc.*, 118:287-288 (1996); Cwirla, et al., Peptides On Phage: A Vast Library of Peptides for Identifying Ligands, *Proc. Natl. Acad. Sci. U.S.A.*, 87:6378-6382 (1990); Ecker, et al., Combinatorial
- 5 Drug Discovery: Which Method will Produce the Greatest Value, *Bio/Technology*, 13:351-360 (1995); Ellington, et al., *In Vitro* Selection of RNA Molecules That Bind Specific Ligands, *Nature*, 346:818-822 (1990); Ellman, J.A., Variants of Benzodiazepines, *J. Am. Chem. Soc.*, 114:10997 (1992); Erickson, et al., *The Proteins*; Neurath, H., Hill, R.L., Eds.: Academic: New York,
- 10 1976; pp. 255-257; Felici, et al., *J. Mol. Biol.*, 222:301-310 (1991); Fodor, et al., Light-Directed, Spatially Addressable Parallel Chemical Synthesis, *Science*, 251:767-773 (1991); Francisco, et al., Transport and Anchoring of Beta-Lactamase to the External Surface of *E. Coli.*, *Proc. Natl. Acad. Sci. U.S.A.*, 89:2713-2717 (1992); Georgiou, et al., Practical Applications of Engineering
- 15 Gram-Negative Bacterial Cell Surfaces, *TIBTECH*, 11:6-10 (1993); Geysen, et al., Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid, *Proc. Natl. Acad. Sci. U.S.A.*, 81:3998-4002 (1984); Glaser, et al., Antibody Engineering by Condon-Based Mutagenesis in a Filamentous Phage Vector System, *J. Immunol.*, 149:3903-3913 (1992); Gram, et al., In
- 20 vitro selection and affinity maturation of antibodies from a naive combinatorial immunoglobulin library, *Proc. Natl. Acad. Sci.*, 89:3576-3580 (1992); Han, et al., Liquid-Phase Combinatorial Synthesis, *Proc. Natl. Acad. Sci. U.S.A.*, 92:6419-6423 (1995); Hoogenboom, et al., Multi-Subunit Proteins on the Surface of Filamentous Phage: Methodologies for Displaying Antibody (Fab)
- 25 Heavy and Light Chains, *Nucleic Acids Res.*, 19:4133-4137 (1991); Houghten, et al., General Method for the Rapid Solid-Phase Synthesis of Large Numbers of Peptides: Specificity of Antigen-Antibody Interaction at the Level of Individual Amino Acids, *Proc. Natl. Acad. Sci. U.S.A.*, 82:5131-5135 (1985); Houghten, et al., The Use of Synthetic Peptide Combinatorial Libraries for the Determination
- 30 of Peptide Ligands in Radio-Receptor Assays-Opioid-Peptides, *Bioorg. Med. Chem. Lett.*, 3:405-412 (1993); Houghten, et al., Generation and Use of Synthetic Peptide Combinatorial Libraries for Basic Research and Drug Discovery,

- Nature*, 354:84-86 (1991); Huang, et al., Discovery of New Ligand Binding Pathways in Myoglobin by Random Mutagenesis, *Nature Struct. Biol.*, 1:226-229 (1994); Huse, et al., Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire In Phage Lambda, *Science*, 246:1275-1281 (1989);
- 5 Janda, K.D., New Strategies for the Design of Catalytic Antibodies, *Biotechnol. Prog.*, 6:178-181 (1990); Jung, et al., Multiple Peptide Synthesis Methods and Their Applications, *Angew. Chem. Int. Ed. Engl.*, 31:367-486 (1992); Kang, et al., Linkage of Recognition and Replication Functions By Assembling Combinatorial Antibody Fab Libraries Along Phage Surfaces, *Proc. Natl. Acad.*
- 10 *Sci. U.S.A.*, 88:4363-4366 (1991a); Kang, et al., Antibody Redesign by Chain Shuffling from Random Combinatorial Immunoglobulin Libraries, *Proc. Natl. Acad. Sci. U.S.A.*, 88:11120-11123 (1991b); Kay, et al., An M13 Phage Library Displaying Random 38-Amino-Acid-Peptides as a Source of Novel Sequences with Affinity to Selected Targets Genes, *Gene*, 128:59-65 (1993); Lam, et al., A
- 15 new type of synthetic peptide library for identifying ligand-binding activity, *Nature*, 354:82-84 (1991) (published errata appear in *Nature*, 358:434 (1992) and *Nature*, 360:768 (1992); Lebl, et al., One Bead One Structure Combinatorial Libraries, *Biopolymers (Pept. Sci.)*, 37:177-198 (1995); Lerner, et al., Antibodies without Immunization, *Science*, 258:1313-1314 (1992); Li, et al., Minimization
- 20 of a Polypeptide Hormone, *Science*, 270:1657-1660 (1995); Light, et al., Display of Dimeric Bacterial Alkaline Phosphatase on the Major Coat Protein of Filamentous Bacteriophage, *Bioorg. Med. Chem. Lett.*, 3:1073-1079 (1992); Little, et al., Bacterial Surface Presentation of Proteins and Peptides: An Alternative to Phage Technology, *Trends Biotechnol.*, 11:3-5 (1993); Marks, et
- 25 al., By-Passing Immunization. Human Antibodies from V-Gene Libraries Displayed on Phage, *J. Mol. Biol.*, 222:581-597 (1991); Matthews, et al., Substrate Phage: Selection of Protease Substrates by Monovalent Phage Display, *Science*, 260:1113-1117 (1993); McCafferty, et al., Phage Enzymes: Expression and Affinity Chromatography of Functional Alkaline Phosphatase on the Surface
- 30 of Bacteriophage, *Protein Eng.*, 4:955-961 (1991); Menger, et al., Phosphatase Catalysis Developed Via Combinatorial Organic Chemistry, *J. Org. Chem.*, 60:6666-6667 (1995); Nicolaou, et al., *Angew. Chem. Int. Ed. Engl.*, 34:2289-

-87-

- 2291 (1995); Oldenburg, et al., Peptide Ligands for A Sugar-Binding Protein Isolated from a Random Peptide Library, *Proc. Natl. Acad. Sci. U.S.A.*, 89:5393-5397 (1992); Parmley, et al., Antibody-Selectable Filamentous fd Phage Vectors: Affinity Purification of Target Genes, *Genes*, 73:305-318 (1988); Pinilla, et al.,
- 5 Synthetic Peptide Combinatorial Libraries (SPCLS)--Identification of the Antigenic Determinant of Beta-Endorphin Recognized by Monoclonal Antibody-3E7, *Gene*, 128:71-76 (1993); Pinilla, et al., Review of the Utility of Soluble Combinatorial Libraries, *Biopolymers*, 37:221-240 (1995); Pistor, et al., Expression of Viral Hemagglutinin On the Surface of *E. Coli*, *Klin. Wochenschr.*, 66:110-116
- 10 (1989); Pollack, et al., Selective Chemical Catalysis by an Antibody, *Science*, 234:1570-1572 (1986); Rigler, et al., Fluorescence Correlations, Single Molecule Detection and Large Number Screening: Applications in Biotechnology, *J. Biotechnol.*, 41:177-186 (1995); Sarvetnick, et al., Increasing the Chemical Potential of the Germ-Line Antibody Repertoire, *Proc. Natl. Acad. Sci. U.S.A.*,
- 15 90:4008-4011 (1993); Sastry, et al., Cloning of the Immunological Repertoire in *Escherichia Coli* for Generation of Monoclonal Catalytic Antibodies: Construction of a Heavy Chain Variable Region-Specific cDNA Library, *Proc. Natl. Acad. Sci. U.S.A.*, 86:5728-5732 (1989); Scott, et al., Searching for Peptide Ligands with an Epitope Library, *Science*, 249:386-390 (1990); Sears, et al., Engineering
- 20 Enzymes for Bioorganic Synthesis: Peptide Bond Formation, *Biotechnol. Prog.*, 12:423-433 (1996); Simon, et. al., Peptides: A Modular Approach to Drug Discovery, *Proc. Natl. Acad. Sci. U.S.A.*, 89:9367-9371 (1992); Still, et al., Discovery of Sequence-Selective Peptide Binding by Synthetic Receptors Using Encoded Combinatorial Libraries, *Acc. Chem. Res.*, 29:155-163 (1996);
- 25 Thompson, et al., Synthesis and Applications of Small Molecule Libraries, *Chem. Rev.*, 96:555-600 (1996); Tramontano, et al., Catalytic Antibodies, *Science*, 234:1566-1570 (1986); Wrighton, et al., Small Peptides as Potent Mimetics of the Protein Hormone Erythropoietin, *Science*, 273:458-464 (1996); York, et al., Combinatorial mutagenesis of the reactive site region in plasminogen activator
- 30 inhibitor I, *J. Biol. Chem.*, 266:8595-8600 (1991); Zebedee, et al., Human Combinatorial Antibody Libraries to Hepatitis B Surface Antigen, *Proc. Natl. Acad. Sci. U.S.A.*, 89:3175-3179 (1992); Zuckerman, et al., Identification of

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Highest-Affinity Ligands by Affinity Selection from Equimolar Peptide Mixtures Generated by Robotic Synthesis, *Proc. Natl. Acad. Sci. U.S.A.*, 89:4505-4509 (1992).

For example, peptides that bind to a CVSP14 polypeptide or a protease domain of an SP protein can be identified using phage display libraries. In an exemplary embodiment, this method can include a) contacting phage from a phage library with the CVSP14 polypeptide or a protease domain thereof; (b) isolating phage that bind to the protein; and (c) determining the identity of at least one peptide coded by the isolated phage to identify a peptide that binds to a CVSP14 polypeptide.

H. Modulators of the activity of CVSP14 polypeptides

Provided herein are compounds, identified by screening or produced using the CVSP14 polypeptide or protease domain in other screening methods, that modulate the activity of a CVSP14. These compounds act by directly interacting with the CVSP14 polypeptide or by altering transcription or translation thereof. Such molecules include, but are not limited to, antibodies that specifically react with a CVSP14 polypeptide, particularly with the protease domain thereof, antisense nucleic acids or double-stranded RNA (dsRNA) such as RNAi, that alter expression of the CVSP14 polypeptide, antibodies, peptide mimetics and other such compounds.

1. Antibodies

Antibodies, including polyclonal and monoclonal antibodies, that specifically bind to the CVSP14 polypeptide provided herein, particularly to the single chain protease domains thereof or the activated forms of the full-length or protease domain or the zymogen form, are provided.

Generally, the antibody is a monoclonal antibody, and typically the antibody specifically binds to the protease domain of the CVSP14 polypeptide. In particular embodiments, antibodies to each of the single chain of the protease domain of CVSP14 are provided. Also provided are antibodies that specifically bind to any domain of CVSP14 and to two chain forms thereof.

The CVSP14 polypeptide and domains, fragments, homologs and derivatives thereof can be used as immunogens to generate antibodies that

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specifically bind such immunogens. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library. In a specific embodiment, antibodies to human CVSP14 polypeptide are produced. In another embodiment, complexes formed from
5 fragments of CVSP14 polypeptide, which fragments contain the serine protease domain, are used as immunogens for antibody production.

Various procedures known in the art can be used for the production of polyclonal antibodies to CVSP14 polypeptide, its domains, derivatives, fragments or analogs. For production of the antibody, various host animals can be
10 immunized by injection with the native CVSP14 polypeptide or a synthetic version, or a derivative of the foregoing, such as a cross-linked CVSP14 polypeptide. Such host animals include but are not limited to rabbits, mice, rats, etc. Various adjuvants can be used to increase the immunological response, depending on the host species, and include but are not limited to Freund's
15 (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, and potentially useful human adjuvants such as bacille Calmette-Guerin (BCG) and corynebacterium parvum.

For preparation of monoclonal antibodies directed towards a CVSP14
20 polypeptide or domains, derivatives, fragments or analogs thereof, any technique that provides for the production of antibody molecules by continuous cell lines in culture can be used. Such techniques include but are not restricted to the hybridoma technique originally developed by Kohler and Milstein (*Nature* 256:495-497 (1975)), the trioma technique, the human B-cell hybridoma
25 technique (Kozbor et al., *Immunology Today* 4:72 (1983)), and the EBV hybridoma technique to produce human monoclonal antibodies (Cole et al., in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 (1985)). In an additional embodiment, monoclonal antibodies can be produced in germ-free animals utilizing recent technology (PCT/US90/02545). Human
30 antibodies can be used and can be obtained by using human hybridomas (Cote et al., *Proc. Natl. Acad. Sci. USA* 80:2026-2030 (1983)), or by transforming human B cells with EBV virus *in vitro* (Cole et al., in *Monoclonal Antibodies and*

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Cancer Therapy, Alan R. Liss, Inc., pp. 77-96 (1985)). Techniques developed for the production of "chimeric antibodies" (Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984); Neuberger et al., *Nature* 312:604-608 (1984); Takeda et al., *Nature* 314:452-454 (1985)) by splicing the genes from a mouse
5 antibody molecule specific for the CVSP14 polypeptide together with genes from a human antibody molecule of appropriate biological activity can be used.

Techniques described for the production of single chain antibodies (U.S. patent 4,946,778) can be adapted to produce CVSP14 polypeptide-specific single chain antibodies. An additional embodiment uses the techniques
10 described for the construction of Fab expression libraries (Huse et al., *Science* 246:1275-1281 (1989)) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for CVSP14 polypeptide or domains, derivatives, or analogs thereof. Non-human antibodies can be "humanized" by known methods (see, e.g., U.S. Patent No. 5,225,539).

15 Antibody fragments that specifically bind to CVSP14 polypeptide or epitopes thereof can be generated by techniques known in the art. For example, such fragments include but are not limited to: the F(ab')₂ fragment, which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments that can be generated by reducing the disulfide bridges of the F(ab')₂ fragment,
20 the Fab fragments that can be generated by treating the antibody molecular with papain and a reducing agent, and Fv fragments.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, e.g., ELISA (enzyme-linked immunosorbent assay). To select antibodies specific to a particular domain of
25 the CVSP14 polypeptide one can assay generated hybridomas for a product that binds to the fragment of the CVSP14 polypeptide that contains such a domain

The foregoing antibodies can be used in methods known in the art relating to the localization and/or quantitation of CVSP14 polypeptide proteins, e.g., for imaging these proteins, measuring levels thereof in appropriate
30 physiological samples, in, for example, diagnostic methods. In another embodiment, anti-CVSP14 polypeptide antibodies, or fragments thereof, containing the binding domain are used as therapeutic agents.

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2. Peptides, Polypeptides and Peptide Mimetics

Provided herein are methods for identifying molecules that bind to and modulate the activity of SP proteins. Included among molecules that bind to SPs, particularly the single chain protease domain or catalytically active fragments thereof, are peptides, polypeptides and peptide mimetics, including cyclic peptides. Peptide mimetics are molecules or compounds that mimic the necessary molecular conformation of a ligand or polypeptide for specific binding to a target molecule such as a CVSP14 polypeptide. In an exemplary embodiment, the peptides, polypeptides and peptide mimetics or peptide mimetics bind to the protease domain of the CVSP14 polypeptide. Such peptides and peptide mimetics include those of antibodies that specifically bind to a CVSP14 polypeptide and, typically, bind to the protease domain of a CVSP14 polypeptide. The peptides, polypeptides and peptide mimetics and peptide mimetics identified by methods provided herein can be agonists or antagonists of CVSP14 polypeptides.

Such peptides and peptide mimetics are useful for diagnosing, treating, preventing, and screening for a disease or disorder associated with CVSP14 polypeptide activity in a mammal. In addition, the peptides and peptide mimetics are useful for identifying, isolating, and purifying molecules or compounds that modulate the activity of a CVSP14 polypeptide, or specifically bind to a CVSP14 polypeptide, generally the protease domain of a CVSP14 polypeptide. Low molecular weight peptides and peptide mimetics can have strong binding properties to a target molecule, *e.g.*, a CVSP14 polypeptide or the protease domain of a CVSP14 polypeptide.

Peptides, polypeptides and peptide mimetics that bind to CVSP14 polypeptides as described herein can be administered to mammals, including humans, to modulate CVSP14 polypeptide activity. Thus, methods for therapeutic treatment and prevention of neoplastic diseases comprise administering a peptide, polypeptides or peptide mimetic compound in an amount sufficient to modulate such activity are provided. Thus, also provided herein are methods for treating a subject having such a disease or disorder in which a

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peptide, polypeptides or peptide mimetic compound is administered to the subject in a therapeutically effective dose or amount.

Compositions containing the peptides, polypeptides or peptide mimetics can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, compositions can be administered to a patient already suffering from a disease, as described above, in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications. Amounts effective for this use will depend on the severity of the disease and the weight and general state of the patient and can be empirically determined.

10 In prophylactic applications, compositions containing the peptides, polypeptides and peptide mimetics are administered to a patient susceptible to or otherwise at risk of a particular disease. Such an amount is defined to be a "prophylactically effective dose". In this use, the precise amounts again depend on the patient's state of health and weight. Accordingly, the peptides, polypeptides and peptide mimetics that bind to a CVSP14 polypeptide can be used to prepare pharmaceutical compositions containing, as an active ingredient, at least one of the peptides or peptide mimetics in association with a pharmaceutical carrier or diluent. The compounds can be administered, for example, by oral, pulmonary, parental (intramuscular, intraperitoneal, intravenous 20 (IV) or subcutaneous injection), inhalation (via a fine powder formulation), transdermal, nasal, vaginal, rectal, or sublingual routes of administration and can be formulated in dosage forms appropriate for each route of administration (see, e.g., International PCT application Nos. WO 93/25221 and WO 94/17784; and European Patent Application 613,683).

25 Peptides, polypeptides and peptide mimetics that bind to CVSP14 polypeptides are useful *in vitro* as unique tools for understanding the biological role of CVSP14 polypeptides, including the evaluation of the many factors thought to influence, and be influenced by, the production of CVSP14 polypeptide. Such peptides, polypeptides and peptide mimetics are also useful in the development of other compounds that bind to and modulate the activity of a 30 CVSP14 polypeptide, because such compounds provide important information on

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the relationship between structure and activity that should facilitate such development.

The peptides, polypeptides and peptide mimetics are also useful as competitive binders in assays to screen for new CVSP14 polypeptides or

- 5 CVSP14 polypeptide agonists. In such assay embodiments, the compounds can be used without modification or can be modified in a variety of ways; for example, by labeling, such as covalently or non-covalently joining a moiety which directly or indirectly provides a detectable signal. In any of these assays, the materials thereto can be labeled either directly or indirectly. Possibilities for
- 10 direct labeling include label groups such as: radiolabels such as ¹²⁵I enzymes (U.S. Pat. No. 3,645,090) such as peroxidase and alkaline phosphatase, and fluorescent labels (U.S. Pat. No. 3,940,475) capable of monitoring the change in fluorescence intensity, wavelength shift, or fluorescence polarization. Possibilities for indirect labeling include biotinylation of one constituent followed
- 15 by binding to avidin coupled to one of the above label groups. The compounds can also include spacers or linkers in cases where the compounds are to be attached to a solid support.

- Moreover, based on their ability to bind to a CVSP14 polypeptide, the peptides, polypeptides and peptide mimetics can be used as reagents for
- 20 detecting CVSP14 polypeptides in living cells, fixed cells, in biological fluids, in tissue homogenates and in purified, natural biological materials. For example, by labelling such peptides, polypeptides and peptide mimetics, cells having CVSP14 polypeptides can be identified. In addition, based on their ability to bind a CVSP14 polypeptide, the peptides, polypeptides and peptide mimetics can
- 25 be used in *in situ* staining, FACS (fluorescence-activated cell sorting), Western blotting, ELISA and other analytical protocols. Based on their ability to bind to a CVSP14 polypeptide, the peptides, polypeptides and peptide mimetics can be used in purification of CVSP14 polypeptide polypeptides or in purifying cells expressing the CVSP14 polypeptide polypeptides, *e.g.*, a polypeptide encoding
- 30 the protease domain of a CVSP14 polypeptide.

The peptides, polypeptides and peptide mimetics can also be used as commercial reagents for various medical research and diagnostic uses. The

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activity of the peptides and peptide mimetics can be evaluated either *in vitro* or *in vivo* in one of the numerous models described in McDonald (1992) *Am. J. of Pediatric Hematology/Oncology*, 14:8-21.

3. Peptide, polypeptides and peptide mimetic therapy

5 Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compounds are termed "peptide mimetics" or "peptidomimetics" (Luthman *et al.*, *A Textbook of Drug Design and Development*, 14:386-406, 2nd Ed., Harwood Academic Publishers (1996);
10 Joachim Grante (1994) *Angew. Chem. Int. Ed. Engl.*, 33:1699-1720; Fauchere (1986) *J. Adv. Drug Res.*, 15:29; Veber and Freidinger (1985) *TINS*, p. 392; and Evans *et al.* (1987) *J. Med. Chem.* 30:1229). Peptide mimetics that are structurally similar to therapeutically useful peptides can be used to produce an equivalent or enhanced therapeutic or prophylactic effect. Preparation of
15 peptidomimetics and structures thereof are known to those of skill in this art.

Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (*e.g.*, D-lysine in place of L-lysine) can be used to generate more stable peptides. In addition, constrained peptides containing a consensus sequence or a substantially identical consensus
20 sequence variation can be generated by methods known in the art (Rizo *et al.* (1992) *An. Rev. Biochem.*, 61:387, incorporated herein by reference); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

Those skilled in the art appreciate that modifications can be made to the
25 peptides and mimetics without deleteriously effecting the biological or functional activity of the peptide. Further, the skilled artisan would know how to design non-peptide structures in three dimensional terms, that mimic the peptides that bind to a target molecule, *e.g.*, a CVSP14 polypeptide or, generally, the protease domain of CVSP14 polypeptides (see, *e.g.*, Eck and Sprang (1989) *J. Biol.*
30 *Chem.*, 26: 17605-18795).

When used for diagnostic purposes, the peptides and peptide mimetics can be labeled with a detectable label and, accordingly, the peptides and peptide

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mimetics without such a label can serve as intermediates in the preparation of labeled peptides and peptide mimetics. Detectable labels can be molecules or compounds, which when covalently attached to the peptides and peptide mimetics, permit detection of the peptide and peptide mimetics *in vivo*, for example, in a patient to whom the peptide or peptide mimetic has been administered, or *in vitro*, e.g., in a sample or cells. Suitable detectable labels are well known in the art and include, by way of example, radioisotopes, fluorescent labels (e.g., fluorescein), and the like. The particular detectable label employed is not critical and is selected to be detectable at non-toxic levels. Selection of the such labels is well within the skill of the art.

Covalent attachment of a detectable label to the peptide or peptide mimetic is accomplished by conventional methods well known in the art. For example, when the ^{125}I radioisotope is employed as the detectable label, covalent attachment of ^{125}I to the peptide or the peptide mimetic can be achieved by incorporating the amino acid tyrosine into the peptide or peptide mimetic and then iodinating the peptide (see, e.g., Weaner *et al.* (1994) *Synthesis and Applications of Isotopically Labelled Compounds*, pp. 137-140). If tyrosine is not present in the peptide or peptide mimetic, incorporation of tyrosine to the N or C terminus of the peptide or peptide mimetic can be achieved by well known chemistry. Likewise, ^{32}P can be incorporated onto the peptide or peptide mimetic as a phosphate moiety through, for example, a hydroxyl group on the peptide or peptide mimetic using conventional chemistry.

Labeling of peptidomimetics usually involves covalent attachment of one or more labels, directly or through a spacer (e.g., an amide group), to non-interfering position(s) on the peptidomimetic that are predicted by quantitative structure-activity data and/or molecular modeling. Such non-interfering positions generally are positions that do not form direct contacts with the macromolecules(s) to which the peptidomimetic binds to produce the therapeutic effect. Derivatization (e.g., labeling) of peptidomimetics should not substantially interfere with the desired biological or pharmacological activity of the peptidomimetic.

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Peptides, polypeptides and peptide mimetics that can bind to a CVSP14 polypeptide or the protease domain of CVSP14 polypeptides and/or modulate the activity thereof, or exhibit CVSP14 polypeptide activity, can be used for treatment of neoplastic disease. The peptides, polypeptides and peptide
5 mimetics can be delivered, *in vivo* or *ex vivo*, to the cells of a subject in need of treatment. Further, peptides which have CVSP14 polypeptide activity can be delivered, *in vivo* or *ex vivo*, to cells which carry mutant or missing alleles encoding the CVSP14 polypeptide gene. Any of the techniques described herein or known to the skilled artisan can be used for preparation and *in vivo* or *ex vivo*
10 delivery of such peptides, polypeptides and peptide mimetics that are substantially free of other human proteins. For example, the peptides, polypeptides and peptide mimetics can be readily prepared by expression in a microorganism or synthesis *in vitro*.

The peptides or peptide mimetics can be introduced into cells, *in vivo* or
15 *ex vivo*, by microinjection or by use of liposomes, for example. Alternatively, the peptides, polypeptides or peptide mimetics can be taken up by cells, *in vivo* or *ex vivo*, actively or by diffusion. In addition, extracellular application of the peptide, polypeptides or peptide mimetic can be sufficient to effect treatment of a neoplastic disease. Other molecules, such as drugs or organic compounds,
20 that: 1) bind to a CVSP14 polypeptide or protease domain thereof; or 2) have a similar function or activity to an CVSP14 polypeptide or protease domain thereof, can be used in methods for treatment.

4. Rational drug design

The goal of rational drug design is to produce structural analogs of
25 biologically active polypeptides or peptides of interest or of small molecules or peptide mimetics with which they interact (*e.g.*, agonists and antagonists) in order to fashion drugs which are, *e.g.*, more active or stable forms thereof; or which, for example, enhance or interfere with the function of a polypeptide *in vivo* (*e.g.*, a CVSP14 polypeptide). In one approach, one first determines the
30 three-dimensional structure of a protein of interest (*e.g.*, a CVSP14 polypeptide or polypeptide having a protease domain) or, for example, of a CVSP14 polypeptide-ligand complex, by X-ray crystallography, by computer modeling or

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most typically, by a combination of approaches (see, *e.g.*, Erickson *et al.* 1990). Also, useful information regarding the structure of a polypeptide can be gained by modeling based on the structure of homologous proteins. In addition, peptides can be analyzed by an alanine scan. In this technique, an amino acid
5 residue is replaced by Ala, and its effect on the peptide's activity is determined. Each of the amino acid residues of the peptide is analyzed in this manner to determine the important regions of the peptide.

Also, a polypeptide or peptide that binds to a CVSP14 polypeptide or, generally, the protease domain of a CVSP14 polypeptide, can be selected by a
10 functional assay, and then the crystal structure of this polypeptide or peptide can be determined. The polypeptide can be, for example, an antibody specific for a CVSP14 polypeptide or the protein domain of a CVSP14 polypeptide. This approach can yield a pharmacophore upon which subsequent drug design can be based. Further, it is possible to bypass the crystallography altogether by
15 generating anti-idiotypic polypeptides or peptides, (anti-ids) to a functional, pharmacologically active polypeptide or peptide that binds to a CVSP14 polypeptide or protease domain of a CVSP14 polypeptide. As a mirror image of a mirror image, the binding site of the anti-ids is expected to be an analog of the original target molecule, *e.g.*, a CVSP14 polypeptide or polypeptide having a
20 CVSP14 polypeptide. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced banks of peptides. Selected peptides would then act as the pharmacophore.

Thus, one can design drugs which have, *e.g.*, improved activity or stability or which act as modulators (*e.g.*, inhibitors, agonists, antagonists) of
25 CVSP14 polypeptide activity, and are useful in the methods, particularly the methods for diagnosis, treatment, prevention, and screening of a neoplastic disease. By virtue of the availability of cloned CVSP14 polypeptide sequences, sufficient amounts of the CVSP14 polypeptide polypeptide can be made available to perform such analytical studies as X-ray crystallography. In addition,
30 the knowledge of the amino acid sequence of a CVSP14 polypeptide or the protease domain thereof, *e.g.*, the protease domain encoded by the amino acid

sequence of SEQ ID Nos. 5 and 6, can provide guidance on computer modeling techniques in place of, or in addition to, X-ray crystallography.

Methods of identifying peptides and peptide mimetics that bind to CVSP14 polypeptides

5 Peptides having a binding affinity to the CVSP14 polypeptide polypeptides provided herein (*e.g.*, a CVSP14 polypeptide or a polypeptide having a protease domain of a CVSP14 polypeptide) can be readily identified, for example, by random peptide diversity generating systems coupled with an affinity enrichment process. Specifically, random peptide diversity generating
10 systems include the "peptides on plasmids" system (see, *e.g.*, U.S. Patent Nos. 5,270,170 and 5,338,665); the "peptides on phage" system (see, *e.g.*, U.S. Patent No. 6,121,238 and Cwirla, *et al.* (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87:6378-6382); the "polysome system;" the "encoded synthetic library (ESL)" system; and the "very large scale immobilized polymer synthesis" system (see,
15 *e.g.*, U.S. Patent No. 6,121,238; and Dower *et al.* (1991) *An. Rep. Med. Chem.* 26:271-280

For example, using the procedures described above, random peptides can generally be designed to have a defined number of amino acid residues in length (*e.g.*, 12). To generate the collection of oligonucleotides encoding the random
20 peptides, the codon motif (NNK)*x*, where N is nucleotide A, C, G, or T (equimolar; depending on the methodology employed, other nucleotides can be employed), K is G or T (equimolar), and *x* is an integer corresponding to the number of amino acids in the peptide (*e.g.*, 12) can be used to specify any one of the 32 possible codons resulting from the NNK motif: 1 for each of 12 amino
25 acids, 2 for each of 5 amino acids, 3 for each of 3 amino acids, and only one of the three stop codons. Thus, the NNK motif encodes all of the amino acids, encodes only one stop codon, and reduces codon bias.

The random peptides can be presented, for example, either on the surface of a phage particle, as part of a fusion protein containing either the pIII or the
30 pVIII coat protein of a phage fd derivative (peptides on phage) or as a fusion protein with the LacI peptide fusion protein bound to a plasmid (peptides on plasmids). The phage or plasmids, including the DNA encoding the peptides, can

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be identified and isolated by an affinity enrichment process using immobilized CVSP14 polypeptide polypeptide having a protease domain. The affinity enrichment process, sometimes called "panning," typically involves multiple rounds of incubating the phage, plasmids, or polysomes with the immobilized
5 CVSP14 polypeptide polypeptide, collecting the phage, plasmids, or polysomes that bind to the CVSP14 polypeptide polypeptide (along with the accompanying DNA or mRNA), and producing more of the phage or plasmids (along with the accompanying LacI-peptide fusion protein) collected.

Characteristics of peptides and peptide mimetics

10 Among the peptides, polypeptides and peptide mimetics for therapeutic application are those of having molecular weights from about 250 to about 8,000 daltons. If such peptides are oligomerized, dimerized and/or derivatized with a hydrophilic polymer (e.g., to increase the affinity and/or activity of the compounds), the molecular weights of such peptides can be substantially greater
15 and can range anywhere from about 500 to about 120,000 daltons, generally from about 8,000 to about 80,000 daltons. Such peptides can contain 9 or more amino acids that are naturally occurring or synthetic (non-naturally occurring) amino acids. One skilled in the art can determine the affinity and molecular weight of the peptides and peptide mimetics suitable for therapeutic
20 and/or diagnostic purposes (e.g., see Dower *et al.*, U.S. Patent No. 6,121,238).

The peptides can be covalently attached to one or more of a variety of hydrophilic polymers. Suitable hydrophilic polymers include, but are not limited to, polyalkylethers as exemplified by polyethylene glycol and polypropylene glycol, polylactic acid, polyglycolic acid, polyoxyalkenes, polyvinylalcohol,
25 polyvinylpyrrolidone, cellulose and cellulose derivatives, dextran and dextran derivatives. When the peptide compounds are derivatized with such polymers, their solubility and circulation half-lives can be increased with little, if any, diminishment in their binding activity. The peptide compounds can be dimerized and each of the dimeric subunits can be covalently attached to a hydrophilic
30 polymer. The peptide compounds can be PEGylated, i.e., covalently attached to polyethylene glycol (PEG).

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5. Methods of preparing peptides and peptide mimetics

Peptides that bind to CVSP14 polypeptides can be prepared by classical methods known in the art, for example, by using standard solid phase techniques. The standard methods include exclusive solid phase synthesis, partial solid phase synthesis methods, fragment condensation, classical solution synthesis, and even by recombinant DNA technology (see, *e.g.*, Merrifield (1963) *J. Am. Chem. Soc.*, 85:2149, incorporated herein by reference.)

Using the "encoded synthetic library" or "very large scale immobilized polymer synthesis" systems (see, *e.g.*, U.S. Patent No. 5,925,525, and 5,902,723); the minimum size of a peptide with the activity of interest can be determined. In addition all peptides that form the group of peptides that differ from the desired motif (or the minimum size of that motif) in one, two, or more residues can be prepared. This collection of peptides then can be screened ability to bind to the target molecule, *e.g.*, and CVSP14 polypeptide or, generally, the protease domain of a CVSP14 polypeptide. This immobilized polymer synthesis system or other peptide synthesis methods can also be used to synthesize truncation analogs and deletion analogs and combinations of truncation and deletion analogs of the peptide compounds.

These procedures can also be used to synthesize peptides in which amino acids other than the 20 naturally occurring, genetically encoded amino acids are substituted at one, two, or more positions of the peptide. For instance, naphthylalanine can be substituted for tryptophan, facilitating synthesis. Other synthetic amino acids that can be substituted into the peptides include L-hydroxypropyl, L-3, 4-dihydroxy-phenylalanyl, D amino acids such as L-d-hydroxylysyl and D-d-methylalanyl, L- α -methylalanyl, β amino acids, and isoquinolyl. D amino acids and non-naturally occurring synthetic amino acids can also be incorporated into the peptides (see, *e.g.*, Roberts *et al.* (1983) *Unusual Amino/Acids in Peptide Synthesis*, 5(6):341-449).

The peptides can also be modified by phosphorylation (see, *e.g.*, W. Bannwarth *et al.* (1996) *Biorganic and Medicinal Chemistry Letters*, 6(17):2141-2146), and other methods for making peptide derivatives (see, *e.g.*,

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Hruby *et al.* (1990) *Biochem. J.*, 268(2):249-262). Thus, peptide compounds also serve as a basis to prepare peptide mimetics with similar biological activity.

Those of skill in the art recognize that a variety of techniques are available for constructing peptide mimetics with the same or similar desired biological activity as the corresponding peptide compound but with more favorable activity than the peptide with respect to solubility, stability, and susceptibility to hydrolysis and proteolysis (see, *e.g.*, Morgan *et al.* (1989) *An. Rep. Med. Chem.*, 24:243-252). Methods for preparing peptide mimetics modified at the N-terminal amino group, the C-terminal carboxyl group, and/or changing one or more of the amido linkages in the peptide to a non-amido linkage are known to those of skill in the art.

Amino terminus modifications include, but are not limited to, alkylating, acetylating and adding a carbobenzoyl group, forming a succinimide group (see, *e.g.*, Murray *et al.* (1995) *Burger's Medicinal Chemistry and Drug Discovery*, 5th ed., Vol. 1, Manfred E. Wolf, ed., John Wiley and Sons, Inc.). C-terminal modifications include mimetics wherein the C-terminal carboxyl group is replaced by an ester, an amide or modifications to form a cyclic peptide.

In addition to N-terminal and C-terminal modifications, the peptide compounds, including peptide mimetics, can advantageously be modified with or covalently coupled to one or more of a variety of hydrophilic polymers. It has been found that when peptide compounds are derivatized with a hydrophilic polymer, their solubility and circulation half-lives can be increased and their immunogenicity is masked, with little, if any, diminishment in their binding activity. Suitable nonproteinaceous polymers include, but are not limited to, polyalkylethers as exemplified by polyethylene glycol and polypropylene glycol, polylactic acid, polyglycolic acid, polyoxyalkenes, polyvinylalcohol, polyvinylpyrrolidone, cellulose and cellulose derivatives, dextran and dextran derivatives. Generally, such hydrophilic polymers have an average molecular weight ranging from about 500 to about 100,000 daltons, including from about 2,000 to about 40,000 daltons and, from about 5,000 to about 20,000 daltons. The hydrophilic polymers also can have an average molecular weights of about 5,000 daltons, 10,000 daltons and 20,000 daltons.

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Methods for derivatizing peptide compounds or for coupling peptides to such polymers have been described (see, *e.g.*, Zallipsky (1995) *Bioconjugate Chem.*, 6:150-165; Monfardini *et al.* (1995) *Bioconjugate Chem.*, 6:62-69; U.S. Pat. No. 4,640,835; U.S. Pat. No. 4,496,689; U.S. Pat. No. 4,301,144; U.S. Pat. No. 4,670,417; U.S. Pat. No. 4,791,192; U.S. Pat. No. 4,179,337 and WO 95/34326, all of which are incorporated by reference in their entirety herein).

Other methods for making peptide derivatives are described, for example, in Hruby *et al.* (1990), *Biochem J.*, 268(2):249-262, which is incorporated herein by reference. Thus, the peptide compounds also serve as structural models for non-peptidic compounds with similar biological activity. Those of skill in the art recognize that a variety of techniques are available for constructing compounds with the same or similar desired biological activity as a particular peptide compound but with more favorable activity with respect to solubility, stability, and susceptibility to hydrolysis and proteolysis (see, *e.g.*, Morgan *et al.* (1989) *An. Rep. Med. Chem.*, 24:243-252, incorporated herein by reference). These techniques include replacing the peptide backbone with a backbone composed of phosphonates, amidates, carbamates, sulfonamides, secondary amines, and N-methylamino acids.

Peptide compounds can exist in a cyclized form with an intramolecular disulfide bond between the thiol groups of the cysteines. Alternatively, an intermolecular disulfide bond between the thiol groups of the cysteines can be produced to yield a dimeric (or higher oligomeric) compound. One or more of the cysteine residues can also be substituted with a homocysteine.

I. Conjugates

A conjugate, containing: a) a single chain protease domain (or proteolytically active portion thereof) of a CVSP14 polypeptide or a full length zymogen, activated form thereof, or two or single chain protease domain thereof; and b) a targeting agent linked to the CVSP14 polypeptide directly or via a linker, wherein the agent facilitates: i) affinity isolation or purification of the conjugate; ii) attachment of the conjugate to a surface; iii) detection of the conjugate; or iv) targeted delivery to a selected tissue or cell, is provided herein. The conjugate can be a chemical conjugate or a fusion protein mixture thereof.

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The targeting agent can be a protein or peptide fragment, such as a tissue specific or tumor specific monoclonal antibody or growth factor or fragment thereof linked either directly or via a linker to a CVSP14 polypeptide or a protease domain thereof. The targeting agent can also be a protein or peptide
5 fragment that contains a protein binding sequence, a nucleic acid binding sequence, a lipid binding sequence, a polysaccharide binding sequence, or a metal binding sequence, or a linker for attachment to a solid support. In a particular embodiment, the conjugate contains a) the CVSP14 or portion thereof, as described herein; and b) a targeting agent linked to the CVSP14 polypeptide
10 directly or via a linker.

Conjugates, such as fusion proteins and chemical conjugates, of the CVSP14 polypeptide with a protein or peptide fragment (or plurality thereof) that functions, for example, to facilitate affinity isolation or purification of the CVSP14 polypeptide domain, attachment of the CVSP14 polypeptide domain to
15 a surface, or detection of the CVSP14 polypeptide domain are provided. The conjugates can be produced by chemical conjugation, such as via thiol linkages, and can be produced by recombinant means as fusion proteins. In the fusion protein, the peptide or fragment thereof is linked to either the N-terminus or C-terminus of the CVSP14 polypeptide domain. In chemical conjugates the peptide
20 or fragment thereof can be linked anywhere that conjugation can be effected, and there can be a plurality of such peptides or fragments linked to a single CVSP14 polypeptide domain or to a plurality thereof.

The targeting agent is for *in vitro* or *in vivo* delivery to a cell or tissue, and includes agents such as cell or tissue-specific antibodies, growth factors and
25 other factors that bind to moieties expressed on specific cells; and other cell or tissue specific agents that promote directed delivery of a linked protein. The targeting agent can be one that specifically delivers the CVSP14 polypeptide to selected cells by interaction with a cell surface protein and internalization of conjugate or CVSP14 polypeptide portion thereof.

30 These conjugates are used in a variety of methods and are particularly suited for use in methods of activation of prodrugs, such as prodrugs that upon cleavage by the particular CVSP14, which is localized at or near the targeted cell

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or tissue, protein are cytotoxic. The prodrugs are administered prior to, or simultaneously with, or subsequently to the conjugate. Upon delivery to the targeted cells, the protease activates the prodrug, which then exhibits a therapeutic effect, such as a cytotoxic effect.

5 **1. Conjugation**

Conjugates with linked CVSP14 polypeptide domains can be prepared either by chemical conjugation, recombinant DNA technology, or combinations of recombinant expression and chemical conjugation. The CVSP14 polypeptide domains and the targeting agent can be linked in any orientation and more than
10 one targeting agents and/or CVSP14 polypeptide domains can be present in a conjugate.

a. Fusion proteins

Fusion proteins are provided herein. A fusion protein contains: a) one or a plurality of domains of a CVSP14 polypeptides and b) a targeting agent. The
15 fusion proteins are generally produced by recombinant expression of nucleic acids that encode the fusion protein.

b. Chemical conjugation

To effect chemical conjugation herein, the CVSP14 polypeptide domain is linked via one or more selected linkers or directly to the targeting agent.

20 Chemical conjugation must be used if the targeted agent is other than a peptide or protein, such a nucleic acid or a non-peptide drug. Any means known to those of skill in the art for chemically conjugating selected moieties can be used.

2. Linkers

Linkers for two purposes are contemplated herein. The conjugates can
25 include one or more linkers between the CVSP14 polypeptide portion and the targeting agent. Additionally, linkers are used for facilitating or enhancing immobilization of a CVSP14 polypeptide or portion thereof on a solid support, such as a microtiter plate, silicon or silicon-coated chip, glass or plastic support, such as for high throughput solid phase screening protocols.

Any linker known to those of skill in the art for preparation of conjugates can be used herein. These linkers are typically used in the preparation of chemical conjugates; peptide linkers can be incorporated into fusion proteins.

Linkers can be any moiety suitable to associate a domain of CVSP14 polypeptide and a targeting agent. Such linkers and linkages include, but are not limited to, peptidic linkages, amino acid and peptide linkages, typically containing between one and about 60 amino acids, more generally between about 10 and 30 amino acids, chemical linkers, such as heterobifunctional cleavable cross-linkers, including but are not limited to, N-succinimidyl (4-iodoacetyl)-aminobenzoate, sulfosuccinimidyl (4-iodoacetyl)-aminobenzoate, 4-succinimidyl-oxy carbonyl-a- (2-pyridyldithio)toluene, sulfosuccinimidyl-6- [a-methyl-a- (pyridyldithio)-toluamido] hexanoate, N-succinimidyl-3-(-2-pyridyldithio) propionate, succinimidyl 6[3-(-2-pyridyldithio)-propionamido] hexanoate, sulfosuccinimidyl 6[3-(-2-pyridyldithio)-propionamido] hexanoate, 3-(2-pyridyldithio)-propionyl hydrazide, Ellman's reagent, dichlorotriazinic acid, and S-(2-thiopyridyl)-L-cysteine. Other linkers include, but are not limited to peptides and other moieties that reduce steric hindrance between the domain of CVSP14 polypeptide and the targeting agent, intracellular enzyme substrates, linkers that increase the flexibility of the conjugate, linkers that increase the solubility of the conjugate, linkers that increase the serum stability of the conjugate, photocleavable linkers and acid cleavable linkers.

Other exemplary linkers and linkages that are suitable for chemically linked conjugates include, but are not limited to, disulfide bonds, thioether bonds, hindered disulfide bonds, and covalent bonds between free reactive groups, such as amine and thiol groups. These bonds are produced using heterobifunctional reagents to produce reactive thiol groups on one or both of the polypeptides and then reacting the thiol groups on one polypeptide with reactive thiol groups or amine groups to which reactive maleimido groups or thiol groups can be attached on the other. Other linkers include, acid cleavable linkers, such as bismaleimideoxy propane, acid labile-transferrin conjugates and adipic acid dihydrazide, that would be cleaved in more acidic intracellular compartments; cross linkers that are cleaved upon exposure to UV or visible

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light and linkers, such as the various domains, such as C_H1, C_H2, and C_H3, from the constant region of human IgG, (see, Batra *et al. Molecular Immunol.*, 30:379-386 (1993)). In some embodiments, several linkers can be included in order to take advantage of desired properties of each linker.

- 5 Chemical linkers and peptide linkers can be inserted by covalently coupling the linker to the domain of CVSP14 polypeptide and the targeting agent. The heterobifunctional agents, described below, can be used to effect such covalent coupling. Peptide linkers can also be linked by expressing DNA encoding the linker and therapeutic agent (TA), linker and targeted agent, or
- 10 linker, targeted agent and therapeutic agent (TA) as a fusion protein. Flexible linkers and linkers that increase solubility of the conjugates are contemplated for use, either alone or with other linkers are also contemplated herein.

a) **Acid cleavable, photocleavable and heat sensitive linkers**

- Acid cleavable linkers, photocleavable and heat sensitive linkers can also
- 15 be used, particularly where it can be necessary to cleave the domain of CVSP14 polypeptide to permit it to be more readily accessible to reaction. Acid cleavable linkers include, but are not limited to, bismaleimideoxy propane; and adipic acid dihydrazide linkers (see, *e.g.*, Fattom *et al. (1992) Infection & Immun.* 60:584-589) and acid labile transferrin conjugates that contain a sufficient
- 20 portion of transferrin to permit entry into the intracellular transferrin cycling pathway (see, *e.g.*, Welhöner *et al. (1991) J. Biol. Chem.* 266:4309-4314).

- Photocleavable linkers are linkers that are cleaved upon exposure to light (see, *e.g.*, Goldmacher *et al. (1992) Bioconj. Chem.* 3:104-107, which linkers are herein incorporated by reference), thereby releasing the targeted agent upon
- 25 exposure to light. Photocleavable linkers that are cleaved upon exposure to light are known (see, *e.g.*, Hazum *et al. (1981) in Pept., Proc. Eur. Pept. Symp.*, 16th, Brunfeldt, K (Ed), pp. 105-110, which describes the use of α -nitrobenzyl group as a photocleavable protective group for cysteine; Yen *et al. (1989) Makromol. Chem* 190:69-82, which describes water soluble photocleavable
- 30 copolymers, including hydroxypropylmethacrylamide copolymer, glycine copolymer, fluorescein copolymer and methylrhodamine copolymer; Goldmacher *et al. (1992) Bioconj. Chem.* 3:104-107, which describes a cross-linker

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and reagent that undergoes photolytic degradation upon exposure to near UV light (350 nm); and Senter *et al.* (1985) *Photochem. Photobiol* 42:231-237, which describes nitrobenzyloxycarbonyl chloride cross linking reagents that produce photocleavable linkages), thereby releasing the targeted agent upon exposure to light. Such linkers would have particular use in treating dermatological or ophthalmic conditions that can be exposed to light using fiber optics. After administration of the conjugate, the eye or skin or other body part can be exposed to light, resulting in release of the targeted moiety from the conjugate. Such photocleavable linkers are useful in connection with diagnostic protocols in which it is desirable to remove the targeting agent to permit rapid clearance from the body of the animal.

b) Other linkers for chemical conjugation

Other linkers, include trityl linkers, particularly, derivatized trityl groups to generate a genus of conjugates that provide for release of therapeutic agents at various degrees of acidity or alkalinity. The flexibility thus afforded by the ability to preselect the pH range at which the therapeutic agent is released allows selection of a linker based on the known physiological differences between tissues in need of delivery of a therapeutic agent (see, *e.g.*, U.S. Patent No. 5,612,474). For example, the acidity of tumor tissues appears to be lower than that of normal tissues.

c) Peptide linkers

The linker moieties can be peptides. Peptide linkers can be employed in fusion proteins and also in chemically linked conjugates. The peptide typically has from about 2 to about 60 amino acid residues, for example from about 5 to about 40, or from about 10 to about 30 amino acid residues. The length selected depends upon factors, such as the use for which the linker is included.

Peptide linkers are advantageous when the targeting agent is proteinaceous. For example, the linker moiety can be a flexible spacer amino acid sequence, such as those known in single-chain antibody research. Examples of such known linker moieties include, but are not limited to, peptides, such as $(\text{Gly}_m\text{Ser})_n$ and $(\text{Ser}_m\text{Gly})_n$, in which n is 1 to 6, including 1 to

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4 and 2 to 4, and m is 1 to 6, including 1 to 4, and 2 to 4, enzyme cleavable linkers and others.

Additional linking moieties are described, for example, in Huston *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 85:5879-5883, 1988; Whitlow, M., *et al.*, *Protein Engineering* 6:989-995, 1993; Newton *et al.*, *Biochemistry* 35:545-553, 1996; A. J. Cumber *et al.*, *Bioconj. Chem.* 3:397-401, 1992; Ladurner *et al.*, *J. Mol. Biol.* 273:330-337, 1997; and U.S. Patent. No. 4,894,443. In some embodiments, several linkers can be included in order to take advantage of desired properties of each linker.

10 3. Targeting agents

Any agent that facilitates detection, immobilization, or purification of the conjugate is contemplated for use herein. For chemical conjugates any moiety that has such properties is contemplated; for fusion proteins, the targeting agent is a protein, peptide or fragment thereof that is sufficient to effects the targeting activity. Contemplated targeting agents include those that deliver the CVSP14 polypeptide or portion thereof to selected cells and tissues. Such agents include tumor specific monoclonal antibodies and portions thereof, growth factors, such as FGF, EGF, PDGF, VEGF, cytokines, including chemokines, and other such agents.

20 4. Nucleic acids, plasmids and cells

Isolated nucleic acid fragments encoding fusion proteins are provided. The nucleic acid fragment that encodes the fusion protein includes: a) nucleic acid encoding a protease domain of a CVSP14 polypeptide; and b) nucleic acid encoding a protein, peptide or effective fragment thereof that facilitates: i) affinity isolation or purification of the fusion protein; ii) attachment of the fusion protein to a surface; or iii) detection of the fusion protein. Generally, the nucleic acid is DNA.

Plasmids for replication and vectors for expression that contain the above nucleic acid fragments are also provided. Cells containing the plasmids and vectors are also provided. The cells can be any suitable host including, but are not limited to, bacterial cells, yeast cells, fungal cells, plant cells, insect cell and animal cells. The nucleic acids, plasmids, and cells containing the plasmids can

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be prepared according to methods known in the art including any described herein.

Also provided are methods for producing the above fusion proteins. An exemplary method includes the steps of growing, i.e. culturing the cells so that the proliferate, cells containing a plasmid encoding the fusion protein under conditions whereby the fusion protein is expressed by the cell, and recovering the expressed fusion protein. Methods for expressing and recovering recombinant proteins are well known in the art (*See generally, Current Protocols in Molecular Biology* (1998) § 16, John Wiley & Sons, Inc.) and such methods can be used for expressing and recovering the expressed fusion proteins.

The recovered fusion proteins can be isolated or purified by methods known in the art such as centrifugation, filtration, chromatograph, electrophoresis, immunoprecipitation, etc., or by a combination thereof (*See generally, Current Protocols in Molecular Biology* (1998) § 10, John Wiley & Sons, Inc.). Generally the recovered fusion protein is isolated or purified through affinity binding between the protein or peptide fragment of the fusion protein and an affinity binding moiety. As discussed in the above sections regarding the construction of the fusion proteins, any affinity binding pairs can be constructed and used in the isolation or purification of the fusion proteins. For example, the affinity binding pairs can be protein binding sequences/protein, DNA binding sequences/DNA sequences, RNA binding sequences/RNA sequences, lipid binding sequences/lipid, polysaccharide binding sequences/polysaccharide, or metal binding sequences/metal.

5. Immobilization and supports or substrates therefor

In certain embodiments, where the targeting agents are designed for linkage to surfaces, the CVSP14 polypeptide can be attached by linkage such as ionic or covalent, non-covalent or other chemical interaction, to a surface of a support or matrix material. Immobilization can be effected directly or via a linker. The CVSP14 polypeptide can be immobilized on any suitable support, including, but are not limited to, silicon chips, and other supports described herein and known to those of skill in the art. A plurality of CVSP14 polypeptide or protease domains thereof can be attached to a support, such as an array (i.e.,

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a pattern of two or more) of conjugates on the surface of a silicon chip or other chip for use in high throughput protocols and formats.

It is also noted that the domains of the CVSP14 polypeptide can be linked directly to the surface or via a linker without a targeting agent linked thereto.

5 Hence chips containing arrays of the domains of the CVSP14 polypeptide.

The matrix material or solid supports contemplated herein are generally any of the insoluble materials known to those of skill in the art to immobilize ligands and other molecules, and are those that used in many chemical syntheses and separations. Such supports are used, for example, in affinity
10 chromatography, in the immobilization of biologically active materials, and during chemical syntheses of biomolecules, including proteins, amino acids and other organic molecules and polymers. The preparation of and use of supports is well known to those of skill in this art; there are many such materials and preparations thereof known. For example, naturally-occurring support materials,
15 such as agarose and cellulose, can be isolated from their respective sources, and processed according to known protocols, and synthetic materials can be prepared in accord with known protocols.

The supports are typically insoluble materials that are solid, porous, deformable, or hard, and have any required structure and geometry, including,
20 but not limited to: beads, pellets, disks, capillaries, hollow fibers, needles, solid fibers, random shapes, thin films and membranes. Thus, the item can be fabricated from the matrix material or combined with it, such as by coating all or part of the surface or impregnating particles.

Typically, when the matrix is particulate, the particles are at least about
25 10-2000 μm , but can be smaller or larger, depending upon the selected application. Selection of the matrices is governed, at least in part, by their physical and chemical properties, such as solubility, functional groups, mechanical stability, surface area swelling propensity, hydrophobic or hydrophilic properties and intended use.

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If necessary, the support matrix material can be treated to contain an appropriate reactive moiety. In some cases, the support matrix material already containing the reactive moiety can be obtained commercially. The support matrix material containing the reactive moiety can thereby serve as the matrix support upon which molecules are linked. Materials containing reactive surface moieties such as amino silane linkages, hydroxyl linkages or carboxysilane linkages can be produced by well established surface chemistry techniques involving silanization reactions, or the like. Examples of these materials are those having surface silicon oxide moieties, covalently linked to gamma-amino-propylsilane, and other organic moieties; N-[3-(triethoxysilyl)propyl]phthelamic acid; and bis-(2-hydroxyethyl)aminopropyltriethoxysilane. Exemplary of readily available materials containing amino group reactive functionalities, include, but are not limited to, para-aminophenyltriethoxysilane. Also derivatized polystyrenes and other such polymers are well known and readily available to those of skill in this art (*e.g.*, the Tentagel® Resins are available with a multitude of functional groups, and are sold by Rapp Polymere, Tubingen, Germany; see, U.S. Patent No. 4,908,405 and U.S. Patent No. 5,292,814; *see, also* Butz et al., *Peptide Res.*, 7:20-23 (1994); and Kleine et al., *Immunobiol.*, 190:53-66 (1994)).

These matrix materials include any material that can act as a support matrix for attachment of the molecules of interest. Such materials are known to those of skill in this art, and include those that are used as a support matrix. These materials include, but are not limited to, inorganics, natural polymers, and synthetic polymers, including, but are not limited to: cellulose, cellulose derivatives, acrylic resins, glass, silica gels, polystyrene, gelatin, polyvinyl pyrrolidone, co-polymers of vinyl and acrylamide, polystyrene cross-linked with divinylbenzene and others (*see*, Merrifield, *Biochemistry*, 3:1385-1390 (1964)), polyacrylamides, latex gels, polystyrene, dextran, polyacrylamides, rubber, silicon, plastics, nitrocellulose, celluloses, natural sponges. Of particular interest herein, are highly porous glasses (*see, e.g.*, U.S. Patent No. 4,244,721) and others prepared by mixing a borosilicate, alcohol and water.

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- Synthetic supports include, but are not limited to: acrylamides, dextran-derivatives and dextran co-polymers, agarose-polyacrylamide blends, other polymers and co-polymers with various functional groups, methacrylate derivatives and co-polymers, polystyrene and polystyrene copolymers (*see, e.g.,*
- 5 Merrifield, *Biochemistry*, 3:1385-1390 (1964); Berg et al., in *Innovation Perspect. Solid Phase Synth. Collect. Pap.*, Int. Symp., 1st, Epton, Roger (Ed), pp. 453-459 (1990); Berg et al., *Pept., Proc. Eur. Pept. Symp.*, 20th, Jung, G. et al. (Eds), pp. 196-198 (1989); Berg et al., *J. Am. Chem. Soc.*, 111:8024-8026 (1989); Kent et al., *Isr. J. Chem.*, 17:243-247 (1979); Kent et
- 10 al., *J. Org. Chem.*, 43:2845-2852 (1978); Mitchell et al., *Tetrahedron Lett.*, 42:3795-3798 (1976); U.S. Patent No. 4,507,230; U.S. Patent No. 4,006,117; and U.S. Patent No. 5,389,449). Such materials include those made from polymers and co-polymers such as polyvinylalcohols, acrylates and acrylic acids such as polyethylene-co-acrylic acid, polyethylene-co-methacrylic acid, polyethy-
- 15 lene-co-ethylacrylate, polyethylene-co-methyl acrylate, polypropylene-co-acrylic acid, polypropylene-co-methyl-acrylic acid, polypropylene-co-ethylacrylate, polypropylene-co-methyl acrylate, polyethylene-co-vinyl acetate, polypropylene-co-vinyl acetate, and those containing acid anhydride groups such as polyethylene-co-maleic anhydride and polypropylene-co-maleic anhydride.
- 20 Liposomes have also been used as solid supports for affinity purifications (Powell et al. *Biotechnol. Bioeng.*, 33:173 (1989)).

- Numerous methods have been developed for the immobilization of proteins and other biomolecules onto solid or liquid supports (*see, e.g.,* Mosbach, *Methods in Enzymology*, 44 (1976); Weetall, *Immobilized Enzymes, Antigens, Antibodies, and Peptides*, (1975); Kennedy et al., *Solid Phase Biochemistry, Analytical and Synthetic Aspects*, Scouten, ed., pp. 253-391 (1983); *see, generally*, Affinity Techniques. Enzyme Purification: *Part B. Methods in Enzymology*, Vol. 34, ed. W. B. Jakoby, M. Wilchek, Acad. Press, N.Y. (1974); and Immobilized Biochemicals and Affinity Chromatography,
- 30 *Advances in Experimental Medicine and Biology*, vol. 42, ed. R. Dunlap, Plenum Press, N.Y. (1974)).

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Among the most commonly used methods are absorption and adsorption or covalent binding to the support, either directly or via a linker, such as the numerous disulfide linkages, thioether bonds, hindered disulfide bonds, and covalent bonds between free reactive groups, such as amine and thiol groups, known to those of skill in art (see, e.g., the PIERCE CATALOG, ImmunoTechnology Catalog & Handbook, 1992-1993, which describes the preparation of and use of such reagents and provides a commercial source for such reagents; Wong, *Chemistry of Protein Conjugation and Cross Linking*, CRC Press (1993); see also DeWitt et al., *Proc. Natl. Acad. Sci. U.S.A.*, 90:6909 (1993); Zuckermann et al., *J. Am. Chem. Soc.*, 114:10646 (1992); Kurth et al., *J. Am. Chem. Soc.*, 116:2661 (1994); Ellman et al., *Proc. Natl. Acad. Sci. U.S.A.*, 91:4708 (1994); Sucholeiki, *Tetrahedron Ltr.*, 35:7307 (1994); Su-Sun Wang, *J. Org. Chem.*, 41:3258 (1976); Padwa et al., *J. Org. Chem.*, 41:3550 (1971); and Vedejs et al., *J. Org. Chem.*, 49:575 (1984), which describe photosensitive linkers).

To effect immobilization, a composition containing the protein or other biomolecule is contacted with a support material such as alumina, carbon, an ion-exchange resin, cellulose, glass or a ceramic. Fluorocarbon polymers have been used as supports to which biomolecules have been attached by adsorption (see, U.S. Patent No. 3,843,443; Published International PCT Application WO/86 03840).

J. Prognosis and diagnosis

CVSP14 polypeptide proteins, domains, analogs, and derivatives thereof, and encoding nucleic acids (and sequences complementary thereto), and anti-CVSP14 polypeptide antibodies, can be used in diagnostics, particularly diagnosis of cervical cancer, colon or pancreatic cancers. Such molecules can be used in assays, such as immunoassays, to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders affecting CVSP14 polypeptide expression, or monitor the treatment thereof. For purposes herein, the presence of CVSP14s in body fluids or tumor tissues are of particular interest.

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In particular, such an immunoassay is carried out by a method including contacting a sample derived from a patient with an anti-CVSP14 polypeptide antibody under conditions such that specific binding can occur, and detecting or measuring the amount of any specific binding by the antibody. Such binding of antibody, in tissue sections, can be used to detect aberrant CVSP14 polypeptide localization or aberrant (*e.g.*, increased, decreased or absent) levels of CVSP14 polypeptide. In a specific embodiment, antibody to CVSP14 polypeptide can be used to assay in a patient tissue or serum sample for the presence of CVSP14 polypeptide where an aberrant level of CVSP14 polypeptide is an indication of a diseased condition.

The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays and protein A immunoassays.

CVSP14 polypeptide genes and related nucleic acid sequences and subsequences, including complementary sequences, also can be used in hybridization assays. CVSP14 polypeptide nucleic acid sequences, or subsequences thereof containing about at least 8 nucleotides, generally 14 or 16 or 30 or more, generally less than 1000 or up to 100, contiguous nucleotides can be used as hybridization probes. Hybridization assays can be used to detect, prognose, diagnose, or monitor conditions, disorders, or disease states associated with aberrant changes in CVSP14 polypeptide expression and/or activity as described herein. In particular, such a hybridization assay is carried out by a method by contacting a sample containing nucleic acid with a nucleic acid probe capable of hybridizing to CVSP14 polypeptide encoding DNA or RNA, under conditions such that hybridization can occur, and detecting or measuring any resulting hybridization.

In a specific embodiment, a method of diagnosing a disease or disorder characterized by detecting an aberrant level of a CVSP14 polypeptide in a

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subject is provided herein by measuring the level of the DNA, RNA, protein or functional activity of the CVSP14 polypeptide in a sample derived from the subject, wherein an increase or decrease in the level of the DNA, RNA, protein or functional activity of the CVSP14 polypeptide, relative to the level of the DNA,
5 RNA, protein or functional activity found in an analogous sample not having the disease or disorder indicates the presence of the disease or disorder in the subject.

Kits for diagnostic use are also provided, that contain in one or more containers an anti-CVSP14 polypeptide antibody, and, optionally, a labeled
10 binding partner to the antibody. Alternatively, the anti-CVSP14 polypeptide antibody can be labeled (with a detectable marker, *e.g.*, a chemiluminescent, enzymatic, fluorescent, or radioactive moiety). A kit is also provided that includes in one or more containers a nucleic acid probe capable of hybridizing to SP protein-encoding RNA. In a specific embodiment, a kit can comprise in one
15 or more containers a pair of primers (*e.g.*, each in the size range of 6-30 nucleotides) that are capable of priming amplification [*e.g.*, by polymerase chain reaction (see *e.g.*, Innis et al., 1990, PCR Protocols, Academic Press, Inc., San Diego, CA), ligase chain reaction (see EP 320,308) use of Q β replicase, cyclic probe reaction, or other methods known in the art under appropriate reaction
20 conditions of at least a portion of an SP protein-encoding nucleic acid. A kit can optionally further comprise in a container a predetermined amount of a purified CVSP14 polypeptide or nucleic acid, *e.g.*, for use as a standard or control.

K. Pharmaceutical compositions and modes of administration

1. Components of the compositions

25 Pharmaceutical compositions containing the identified compounds that modulate the activity of a CVSP14 polypeptide are provided herein. Also provided are combinations of a compound that modulates the activity of a CVSP14 polypeptide and another treatment or compound for treatment of a neoplastic disorder, such as a chemotherapeutic compound.

30 The CVSP14 polypeptide modulator and the anti-tumor agent can be packaged as separate compositions for administration together or sequentially or intermittently. Alternatively, they can provided as

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a single composition for administration or as two compositions for administration as a single composition. The combinations can be packaged as kits.

a. CVSP14 polypeptide inhibitors

Any CVSP14 polypeptide inhibitors, including those described herein
5 when used alone or in combination with other compounds, that can alleviate, reduce, ameliorate, prevent, or place or maintain in a state of remission of clinical symptoms or diagnostic markers associated with neoplastic diseases, including undesired and/or uncontrolled angiogenesis, can be used in the present combinations.

10 In one embodiment, the CVSP14 polypeptide inhibitor is an antibody or fragment thereof that specifically reacts with a CVSP14 polypeptide or the protease domain thereof, an inhibitor of the CVSP14 polypeptide production, an inhibitor of CVSP14 polypeptide membrane-localization, or any inhibitor of the expression of or, especially, the activity of a CVSP14 polypeptide.

15 b. Anti-angiogenic agents and anti-tumor agents

Any anti-angiogenic agents and anti-tumor agents, including those described herein, when used alone or in combination with other compounds, that can alleviate, reduce, ameliorate, prevent, or place or maintain in a state of remission of clinical symptoms or diagnostic markers associated with undesired
20 and/or uncontrolled angiogenesis and/or tumor growth and metastasis, particularly solid neoplasms, vascular malformations and cardiovascular disorders, chronic inflammatory diseases and aberrant wound repairs, circulatory disorders, crest syndromes, dermatological disorders, or ocular disorders, can be used in the combinations. Also contemplated are anti-tumor agents for use in
25 combination with an inhibitor of a CVSP14 polypeptide.

c. Anti-tumor agents and anti-angiogenic agents

The compounds identified by the methods provided herein or provided herein can be used in combination with anti-tumor agents and/or anti-angiogenesis agents.

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2. Formulations and route of administration

The compounds herein and agents can be formulated as pharmaceutical compositions, typically for single dosage administration. The concentrations of the compounds in the formulations are effective for delivery of an amount, upon
5 administration, that is effective for the intended treatment. Typically, the compositions are formulated for single dosage administration. To formulate a composition, the weight fraction of a compound or mixture thereof is dissolved, suspended, dispersed or otherwise mixed in a selected vehicle at an effective concentration such that the treated condition is relieved or ameliorated.

10 Pharmaceutical carriers or vehicles suitable for administration of the compounds provided herein include any such carriers known to those skilled in the art to be suitable for the particular mode of administration.

In addition, the compounds can be formulated as the sole
pharmaceutically active ingredient in the composition or can be combined with
15 other active ingredients. Liposomal suspensions, including tissue-targeted liposomes, can also be suitable as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art. For example, liposome formulations can be prepared as described in U.S. Patent No. 4,522,811.

20 The active compound is included in the pharmaceutically acceptable carrier in an amount sufficient to exert a therapeutically useful effect in the absence of undesirable side effects on the patient treated. The therapeutically effective concentration can be determined empirically by testing the compounds in known in vitro and in vivo systems, such as the assays provided herein.

25 The concentration of active compound in the drug composition depends on absorption, inactivation and excretion rates of the active compound, the physicochemical characteristics of the compound, the dosage schedule, and amount administered as well as other factors known to those of skill in the art.

Typically a therapeutically effective dosage is contemplated. The
30 amounts administered can be on the order of 0.001 to 1 mg/ml, including about 0.005-0.05 mg/ml and about 0.01 mg/ml, of blood volume. Pharmaceutical dosage unit forms are prepared to provide from about 1 mg to about 1000 mg,

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including from about 10 to about 500 mg, and including about 25-75 mg of the essential active ingredient or a combination of essential ingredients per dosage unit form. The precise dosage can be empirically determined.

The active ingredient can be administered at once, or can be divided into
5 a number of smaller doses to be administered at intervals of time. It is understood that the precise dosage and duration of treatment is a function of the disease being treated and can be determined empirically using known testing protocols or by extrapolation from in vivo or in vitro test data. It is to be noted that concentrations and dosage values can also vary with the severity of the
10 condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the
15 scope or use of the claimed compositions and combinations containing them.

Pharmaceutically acceptable derivatives include acids, salts, esters, hydrates, solvates and prodrug forms. The derivative is typically selected such that its pharmacokinetic properties are superior to the corresponding neutral compound.

20 Thus, effective concentrations or amounts of one or more of the compounds provided herein or pharmaceutically acceptable derivatives thereof are mixed with a suitable pharmaceutical carrier or vehicle for systemic, topical or local administration to form pharmaceutical compositions. Compounds are included in an amount effective for ameliorating or treating the disorder for
25 which treatment is contemplated. The concentration of active compound in the composition depends on absorption, inactivation, excretion rates of the active compound, the dosage schedule, amount administered, particular formulation as well as other factors known to those of skill in the art.

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Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include any of the following components: a sterile diluent, such as water for injection, saline solution, fixed oil, polyethylene glycol, glycerine, propylene glycol or other synthetic solvent; antimicrobial agents, such as benzyl alcohol and methyl parabens; antioxidants, such as ascorbic acid and sodium bisulfite; chelating agents, such as ethylenediaminetetraacetic acid (EDTA); buffers, such as acetates, citrates and phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose. Parenteral preparations can be enclosed in ampules, disposable syringes or single or multiple dose vials made of glass, plastic or other suitable material.

In instances in which the compounds exhibit insufficient solubility, methods for solubilizing compounds can be used. Such methods are known to those of skill in this art, and include, but are not limited to, using cosolvents, such as dimethylsulfoxide (DMSO), using surfactants, such as Tween®, or dissolution in aqueous sodium bicarbonate. Derivatives of the compounds, such as prodrugs of the compounds can also be used in formulating effective pharmaceutical compositions. For ophthalmic indications, the compositions are formulated in an ophthalmically acceptable carrier. For the ophthalmic uses herein, local administration, either by topical administration or by injection are contemplated. Time release formulations are also desirable. Typically, the compositions are formulated for single dosage administration, so that a single dose administers an effective amount.

Upon mixing or addition of the compound with the vehicle, the resulting mixture can be a solution, suspension, emulsion or other composition. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the compound in the selected carrier or vehicle. If necessary, pharmaceutically acceptable salts or other derivatives of the compounds are prepared.

The compound is included in the pharmaceutically acceptable carrier in an amount sufficient to exert a therapeutically useful effect in the absence of undesirable side effects on the patient treated. It is understood that number and degree of side effects depends upon the condition for which the compounds are

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administered. For example, certain toxic and undesirable side effects are tolerated when treating life-threatening illnesses that would not be tolerated when treating disorders of lesser consequence.

The compounds also can be mixed with other active materials, that do not impair the desired action, or with materials that supplement the desired action known to those of skill in the art. The formulations of the compounds and agents for use herein include those suitable for oral, rectal, topical, inhalational, buccal (*e.g.*, sublingual), parenteral (*e.g.*, subcutaneous, intramuscular, intradermal, or intravenous), transdermal administration or any route. The most suitable route in any given case depends on the nature and severity of the condition being treated and on the nature of the particular active compound which is being used. The formulations are provided for administration to humans and animals in unit dosage forms, such as tablets, capsules, pills, powders, granules, sterile parenteral solutions or suspensions, and oral solutions or suspensions, and oil-water emulsions containing suitable quantities of the compounds or pharmaceutically acceptable derivatives thereof. The pharmaceutically therapeutically active compounds and derivatives thereof are typically formulated and administered in unit-dosage forms or multiple-dosage forms. Unit-dose forms as used herein refers to physically discrete units suitable for human and animal subjects and packaged individually as is known in the art. Each unit-dose contains a predetermined quantity of the therapeutically active compound sufficient to produce the desired therapeutic effect, in association with the required pharmaceutical carrier, vehicle or diluent. Examples of unit-dose forms include ampoules and syringes and individually packaged tablets or capsules. Unit-dose forms can be administered in fractions or multiples thereof. A multiple-dose form is a plurality of identical unit-dosage forms packaged in a single container to be administered in segregated unit-dose form. Examples of multiple-dose forms include vials, bottles of tablets or capsules or bottles of pints or gallons. Hence, multiple dose form is a multiple of unit-doses which are not segregated in packaging.

The composition can contain along with the active ingredient: a diluent such as lactose, sucrose, dicalcium phosphate, or carboxymethylcellulose; a

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lubricant, such as magnesium stearate, calcium stearate and talc; and a binder such as starch, natural gums, such as gum acaciagelatin, glucose, molasses, polvinylpyrrolidine, celluloses and derivatives thereof, povidone, crospovidones and other such binders known to those of skill in the art. Liquid

- 5 pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, or otherwise mixing an active compound as defined above and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, glycols, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be
- 10 administered can also contain minor amounts of nontoxic auxiliary substances such as wetting agents, emulsifying agents, or solubilizing agents, pH buffering agents and the like, for example, acetate, sodium citrate, cyclodextrine derivatives, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and other such agents. Methods of preparing such
- 15 dosage forms are known, or will be apparent, to those skilled in this art (see, *e.g.*, Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., 15th Edition, 1975). The composition or formulation to be administered contains a quantity of the active compound in an amount sufficient to alleviate the symptoms of the treated subject.

- 20 Dosage forms or compositions containing active ingredient in the range of 0.005% to 100% with the balance made up from non-toxic carrier can be prepared. For oral administration, the pharmaceutical compositions can take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*,
- 25 pregelatinized maize starch, polyvinyl pyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets can be coated by methods well-
- 30 known in the art.

The pharmaceutical preparation can also be in liquid form, for example, solutions, syrups or suspensions, or can be presented as a drug product for

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reconstitution with water or other suitable vehicle before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid).

Formulations suitable for rectal administration can be presented as unit dose suppositories. These can be prepared by admixing the active compound with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

Formulations suitable for topical application to the skin or to the eye generally are formulated as an ointment, cream, lotion, paste, gel, spray, aerosol and oil. Carriers which can be used include vaseline, lanoline, polyethylene glycols, alcohols, and combinations of two or more thereof. The topical formulations can further advantageously contain 0.05 to 15 percent by weight of thickeners selected from among hydroxypropyl methyl cellulose, methyl cellulose, polyvinylpyrrolidone, polyvinyl alcohol, poly (alkylene glycols), poly/hydroxyalkyl, (meth)acrylates or poly(meth)acrylamides. A topical formulation is often applied by instillation or as an ointment into the conjunctival sac. It also can be used for irrigation or lubrication of the eye, facial sinuses, and external auditory meatus. It can also be injected into the anterior eye chamber and other places. The topical formulations in the liquid state can be also present in a hydrophilic three-dimensional polymer matrix in the form of a strip, contact lens, and the like from which the active components are released.

For administration by inhalation, the compounds for use herein can be delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin, for use in an inhaler or insufflator can be formulated

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containing a powder mix of the compound and a suitable powder base such as lactose or starch.

Formulations suitable for buccal (sublingual) administration include, for example, lozenges containing the active compound in a flavored base, usually
5 sucrose and acacia or tragacanth; and pastilles containing the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

The compounds can be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, *e.g.*, in ampules or in multi-dose
10 containers, with an added preservative. The compositions can be suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder form for reconstitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water or other solvents, before use.

15 Formulations suitable for transdermal administration can be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably contain the active compound as an optionally buffered aqueous solution of, for example, 0.1 to 0.2 M concentration with respect to the active compound. Formulations
20 suitable for transdermal administration can also be delivered by iontophoresis (*see, e.g.*, *Pharmaceutical Research* 3 (6), 318 (1986)) and typically take the form of an optionally buffered aqueous solution of the active compound.

The pharmaceutical compositions can also be administered by controlled release means and/or delivery devices (*see, e.g.*, in U.S. Patent Nos. 3,536,809;
25 3,598,123; 3,630,200; 3,845,770; 3,847,770; 3,916,899; 4,008,719; 4,687,610; 4,769,027; 5,059,595; 5,073,543; 5,120,548; 5,354,566; 5,591,767; 5,639,476; 5,674,533 and 5,733,566).

Desirable blood levels can be maintained by a continuous infusion of the active agent as ascertained by plasma levels. It should be noted that the
30 attending physician would know how to and when to terminate, interrupt or adjust therapy to lower dosage due to toxicity, or bone marrow, liver or kidney dysfunctions. Conversely, the attending physician would also know how to and

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when to adjust treatment to higher levels if the clinical response is not adequate (precluding toxic side effects).

The efficacy and/or toxicity of the CVSP14 polypeptide inhibitor(s), alone or in combination with other agents also can be assessed by the methods known
5 in the art (See generally, O'Reilly, *Investigational New Drugs*, 15:5-13 (1997)).

The active compounds or pharmaceutically acceptable derivatives can be prepared with carriers that protect the compound against rapid elimination from the body, such as time release formulations or coatings.

Kits containing the compositions and/or the combinations with
10 instructions for administration thereof are provided. The kit can further include a needle or syringe, typically packaged in sterile form, for injecting the complex, and/or a packaged alcohol pad. Instructions are optionally included for administration of the active agent by a clinician or by the patient.

Finally, the compounds or CVSP14 polypeptides or protease domains
15 thereof or compositions containing any of the preceding agents can be packaged as articles of manufacture containing packaging material, a compound or suitable derivative thereof provided herein, which is effective for treatment of a diseases or disorders contemplated herein, within the packaging material, and a label that indicates that the compound or a suitable derivative thereof is for treating the
20 diseases or disorders contemplated herein. The label can optionally include the disorders for which the therapy is warranted.

L. Methods of treatment

The compounds identified by the methods herein are used for treating or preventing neoplastic diseases in an animal, particularly a mammal, including a
25 human, is provided herein. In one embodiment, the method includes administering to a mammal an effective amount of an inhibitor of a CVSP14 polypeptide, whereby the disease or disorder is treated or prevented.

In an embodiment, the CVSP14 polypeptide inhibitor used in the treatment or prevention is administered with a pharmaceutically acceptable
30 carrier or excipient. The mammal treated can be a human. The inhibitors provided herein are those identified by the screening assays. In addition,

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antibodies and antisense nucleic acids or double-stranded RNA (dsRNA), such as RNAi, are contemplated.

The treatment or prevention method can further include administering an anti-angiogenic treatment or agent or anti-tumor agent simultaneously with, prior to or subsequent to the CVSP14 polypeptide inhibitor, which can be any compound identified that inhibits the activity of a CVSP14 polypeptide. Such compounds include small molecule modulators, an antibody or a fragment or derivative thereof containing a binding region thereof against the CVSP14 polypeptide, an antisense nucleic acid or double-stranded RNA (dsRNA), such as RNAi, encoding the CVSP14 polypeptide, and a nucleic acid containing at least a portion of a gene encoding the CVSP14 polypeptide into which a heterologous nucleotide sequence has been inserted such that the heterologous sequence inactivates the biological activity of at least a portion of the gene encoding the CVSP14 polypeptide, in which the portion of the gene encoding the CVSP14 polypeptide flanks the heterologous sequence to promote homologous recombination with a genomic gene encoding the CVSP14 polypeptide. In addition, such molecules are generally less than about 1000 nt long.

1. Antisense treatment

In a specific embodiment, as described hereinabove, CVSP14 polypeptide function is reduced or inhibited by CVSP14 polypeptide antisense nucleic acids, to treat or prevent neoplastic disease. The therapeutic or prophylactic use of nucleic acids of at least six nucleotides that are antisense to a gene or cDNA encoding CVSP14 polypeptide or a portion thereof. A CVSP14 polypeptide "antisense" nucleic acid as used herein refers to a nucleic acid capable of hybridizing to a portion of a CVSP14 polypeptide RNA (generally mRNA) by virtue of some sequence complementarity, and generally under high stringency conditions. The antisense nucleic acid can be complementary to a coding and/or noncoding region of a CVSP14 polypeptide mRNA. Such antisense nucleic acids have utility as therapeutics that reduce or inhibit CVSP14 polypeptide function, and can be used in the treatment or prevention of disorders as described *supra*.

The CVSP14 polypeptide antisense nucleic acids are of at least six nucleotides and are generally oligonucleotides (ranging from 6 to about 150

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nucleotides including 6 to 50 nucleotides). The antisense molecule can be complementary to all or a portion of the protease domain. For example, the oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 125 nucleotides. The oligonucleotides can be DNA or
5 RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide can include other appending groups such as peptides, or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger et al., *Proc. Natl. Acad. Sci. U.S.A.*
10 86:6553-6556 (1989); Lemaitre et al., *Proc. Natl. Acad. Sci. U.S.A.* 84:648-652 (1987); PCT Publication No. WO 88/09810, published December 15, 1988) or blood-brain barrier (see, *e.g.*, PCT Publication No. WO 89/10134, published April 25, 1988), hybridization-triggered cleavage agents (see, *e.g.*, Krol et al., *BioTechniques* 6:958-976 (1988)) or intercalating agents (see, *e.g.*, Zon, *Pharm.*
15 *Res.* 5:539-549 (1988)).

The CVSP14 polypeptide antisense nucleic acid generally is an oligonucleotide, typically single-stranded DNA or RNA or an analog thereof or mixtures thereof. For example, the oligonucleotide includes a sequence antisense to a portion of human CVSP14 polypeptide. The oligonucleotide can
20 be modified at any position on its structure with substituents generally known in the art.

The CVSP14 polypeptide antisense oligonucleotide can include at least one modified base moiety which is selected from the group including, but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil,
25 hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine,
30 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v),

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wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

- 5 In another embodiment, the oligonucleotide includes at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose. The oligonucleotide can include at least one modified phosphate backbone selected from a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a
10 phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

- The oligonucleotide can be an α -anomeric oligonucleotide. An α -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which the strands run parallel to each other (Gautier et al., *Nucl. Acids Res.*
15 15:6625-6641 (1987)).

 The oligonucleotide can be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent and hybridization-triggered cleavage agent.

- The oligonucleotides can be synthesized by standard methods known in
20 the art, *e.g.* by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides can be synthesized by the method of Stein et al. (*Nucl. Acids Res.* 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., *Proc.*
25 *Natl. Acad. Sci. U.S.A.* 85:7448-7451 (1988)), etc.

- In a specific embodiment, the CVSP14 polypeptide antisense oligonucleotide includes catalytic RNA or a ribozyme (see, *e.g.*, PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al., *Science* 247:1222-1225 (1990)). In another embodiment, the oligonucleotide is a 2'-O-
30 methylribonucleotide (Inoue et al., *Nucl. Acids Res.* 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., *FEBS Lett.* 215:327-330 (1987)).

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Alternatively, the oligonucleotide can be double-stranded RNA (dsRNA) such as RNAi.

In an alternative embodiment, the CVSP14 polypeptide antisense nucleic acid is produced intracellularly by transcription from an exogenous sequence.

- 5 For example, a vector can be introduced *in vivo* such that it is taken up by a cell, within which cell the vector or a portion thereof is transcribed, producing an antisense nucleic acid (RNA). Such a vector would contain a sequence encoding the CVSP14 polypeptide antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed
- 10 to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the CVSP14 polypeptide antisense RNA can be by any promoter known in the art to act in mammalian,
- 15 including human, cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, *Nature* 290:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell* 22:787-797 (1980), the herpes thymidine kinase promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster et al., *Nature* 296:39-42 (1982), etc.
- 20

The antisense nucleic acids include sequence complementary to at least a portion of an RNA transcript of a CVSP14 polypeptide gene, including a human CVSP14 polypeptide gene. Absolute complementarity is not required.

- 25 The amount of CVSP14 polypeptide antisense nucleic acid (dsRNA) that is effective in the treatment or prevention of neoplastic disease depends on the nature of the disease, and can be determined empirically by standard clinical techniques. Where possible, it is desirable to determine the antisense cytotoxicity in cells *in vitro*, and then in useful animal model systems prior to
- 30 testing and use in humans.

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2. RNA interference

RNA interference (RNAi) (see, *e.g.* Chuang *et al.* (2000) *Proc. Natl. Acad. Sci. U.S.A.* 97:4985) can be employed to inhibit the expression of a gene encoding a CVSP14. Interfering RNA (RNAi) fragments, particularly double-stranded (ds) RNAi, can be used to generate loss-of-CVSP14 function. Methods relating to the use of RNAi to silence genes in organisms including, mammals, *C. elegans*, *Drosophila* and plants, and humans are known (see, *e.g.*, Fire *et al.* (1998) *Nature* 391:806-811; Fire (1999) *Trends Genet.* 15:358-363; Sharp (2001) *Genes Dev.* 15:485-490; Hammond, *et al.* (2001) *Nature Rev. Genet.* 2:110-1119; Tuschl (2001) *Chem. Biochem.* 2:239-245; Hamilton *et al.* (1999) *Science* 286:950-952; Hammond *et al.* (2000) *Nature* 404:293-296; Zamore *et al.* (2000) *Cell* 101:25-33; Bernstein *et al.* (2001) *Nature* 409: 363-366; Elbashir *et al.* (2001) *Genes Dev.* 15:188-200; Elbashir *et al.* (2001) *Nature* 411:494-498; International PCT application No. WO 01/29058; International PCT application No. WO 99/32619). Double-stranded RNA (dsRNA)-expressing constructs are introduced into a host, such as an animal or plant using, a replicable vector that remains episomal or integrates into the genome. By selecting appropriate sequences, expression of dsRNA can interfere with accumulation of endogenous mRNA encoding a CVSP14. RNAi also can be used to inhibit expression *in vitro*. Regions include at least about 21 (or 21) nucleotides that are selective (i.e. unique) for CVSP14 are used to prepare the RNAi. Smaller fragments of about 21 nucleotides can be transformed directly into cells; larger RNAi dsRNA molecules are generally introduced using vectors that encode them. dsRNA molecules are at least about 21 bp long or longer, such as 50, 100, 150, 200 and longer.

3. Gene Therapy

In an exemplary embodiment, nucleic acids that include a sequence of nucleotides encoding a CVSP14 polypeptide or functional domains or derivative thereof, are administered to promote CVSP14 polypeptide function, by way of gene therapy. Gene therapy refers to therapy performed by the administration of a nucleic acid to a subject. In this embodiment, the nucleic acid produces its encoded protein that mediates a therapeutic effect by promoting CVSP14

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polypeptide function. Any of the methods for gene therapy available in the art can be used (see, Goldspiel et al., *Clinical Pharmacy* 12:488-505 (1993); Wu and Wu, *Biotherapy* 3:87-95 (1991); Tolstoshev, *An. Rev. Pharmacol. Toxicol.* 32:573-596 (1993); Mulligan, *Science* 260:926-932 (1993); and Morgan and
5 Anderson, *An. Rev. Biochem.* 62:191-217 (1993); *TIBTECH* 11(5):155-215 (1993). For example, one therapeutic composition for gene therapy includes a CVSP14 polypeptide-encoding nucleic acid that is part of an expression vector that expresses a CVSP14 polypeptide or domain, fragment or chimeric protein thereof in a suitable host. In particular, such a nucleic acid has
10 a promoter operably linked to the CVSP14 polypeptide coding region, the promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, a nucleic acid molecule is used in which the CVSP14 polypeptide coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in
15 the genome, thus providing for intrachromosomal expression of the SP protein nucleic acid (Koller and Smithies, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); Zijlstra et al., *Nature* 342:435-438 (1989)).

Delivery of the nucleic acid into a patient can be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying
20 vector, or indirect, in which case, cells are first transformed with the nucleic acid *in vitro*, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid is directly administered *in vivo*, where it is expressed to produce the encoded product. This can be
25 accomplished by any of numerous methods known in the art, *e.g.*, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by infection using a defective or attenuated retroviral or other viral vector (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle
30 bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to a peptide

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which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see e.g., Wu and Wu, *J. Biol. Chem.* 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, a nucleic acid-ligand
5 complex can be formed in which the ligand is a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180 dated April 16, 1992 (Wu et al.); WO 92/22635
10 dated December 23, 1992 (Wilson et al.); WO92/20316 dated November 26, 1992 (Findeis et al.); WO93/14188 dated July 22, 1993 (Clarke et al.), WO 93/20221 dated October 14, 1993 (Young)). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); Zijlstra et al., *Nature* 342:435-438 (1989)).

In a specific embodiment, a viral vector that contains the CVSP14 polypeptide nucleic acid is used. For example, a retroviral vector can be used (see Miller et al., *Meth. Enzymol.* 217:581-599 (1993)). These retroviral vectors have been modified to delete retroviral sequences that are not necessary for
20 packaging of the viral genome and integration into host cell DNA. The CVSP14 polypeptide nucleic acid to be used in gene therapy is cloned into the vector, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., *Biotherapy* 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic
25 stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., *J. Clin. Invest.* 93:644-651 (1994); Kiem et al., *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114
30 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory

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epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, 5 *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science* 252:431- 10 434 (1991); Rosenfeld et al., *Cell* 68:143-155 (1992); and Mastrangeli et al., *J. Clin. Invest.* 91:225-234 (1993).

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., *Proc. Soc. Exp. Biol. Med.* 204:289-300 (1993)).

Another approach to gene therapy involves transferring a gene to cells in 15 tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

20 In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, 25 chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see *e.g.*, Loeffler and Behr, *Meth. Enzymol.* 217:599-618 (1993); Cohen et al., *Meth. Enzymol.* 217:618-644 (1993); Cline, *Pharmac. Ther.* 29:69-92 (1985)) and can be used, provided that 30 the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the

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nucleic acid to the cell, so that the nucleic acid is expressible by the cell and generally heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. In an embodiment, epithelial cells are injected, *e.g.*, subcutaneously. In another embodiment, recombinant skin cells can be applied as a skin graft onto the patient. Recombinant blood cells (*e.g.*, hematopoietic stem or progenitor cells) can be administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, *e.g.*, such as stem cells obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, and other sources thereof.

For example, a cell used for gene therapy is autologous to the patient. In an embodiment in which recombinant cells are used in gene therapy, a CVSP14 polypeptide nucleic acid is introduced into the cells such that it is expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment. Such stem cells include but are not limited to hematopoietic stem cells (HSC), stem cells of epithelial tissues such as the skin and the lining of the gut, embryonic heart muscle cells, liver stem cells (PCT Publication WO 94/08598, dated April 28, 1994), and neural stem cells (Stemple and Anderson, *Cell* 71:973-985 (1992)).

Epithelial stem cells (ESCs) or keratinocytes can be obtained from tissues such as the skin and the lining of the gut by known procedures (Rheinwald, *Meth. Cell Bio.* 21A:229 (1980)). In stratified epithelial tissue such as the skin,

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renewal occurs by mitosis of stem cells within the germinal layer, the layer closest to the basal lamina. Stem cells within the lining of the gut provide for a rapid renewal rate of this tissue. ESCs or keratinocytes obtained from the skin or lining of the gut of a patient or donor can be grown in tissue culture

- 5 (Rheinwald, *Meth. Cell Bio.* 21A:229 (1980); Pittelkow and Scott, *Cano Clinic Proc.* 61:771 (1986)). If the ESCs are provided by a donor, a method for suppression of host versus graft reactivity (e.g., irradiation, drug or antibody administration to promote moderate immunosuppression) also can be used.

- With respect to hematopoietic stem cells (HSC), any technique which
- 10 provides for the isolation, propagation, and maintenance *in vitro* of HSC can be used in this embodiment. Techniques by which this can be accomplished include (a) the isolation and establishment of HSC cultures from bone marrow cells isolated from the future host, or a donor, or (b) the use of previously established long-term HSC cultures, which can be allogeneic or xenogeneic.
- 15 Non-autologous HSC generally are used with a method of suppressing transplantation immune reactions of the future host/patient. In a particular embodiment, human bone marrow cells can be obtained from the posterior iliac crest by needle aspiration (see, e.g., Kodo et al., *J. Clin. Invest.* 73:1377-1384 (1984)). For example, the HSCs can be made highly enriched or in substantially
- 20 pure form. This enrichment can be accomplished before, during, or after long-term culturing, and can be done by any techniques known in the art. Long-term cultures of bone marrow cells can be established and maintained by using, for example, modified Dexter cell culture techniques (Dexter et al., *J. Cell Physiol.* 91:335 (1977) or Witlock-Witte culture techniques (Witlock and Witte, *Proc.*
- 25 *Natl. Acad. Sci. USA* 79:3608-3612 (1982)).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy includes an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

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3. Prodrugs

A method for treating tumors is provided. The method is practiced by administering a prodrug that is cleaved at a specific site by a CVSP14 to release an active drug. Upon contact with a cell that expresses CVSP14 activity, the
5 prodrug is converted into an active drug. The prodrug can be a conjugate that contains the active agent, such as an anti-tumor drug, such as a cytotoxic agent, or other therapeutic agent (TA), linked to a substrate for the targeted CVSP14, such that the drug or agent is inactive or unable to enter a cell, in the conjugate, but is activated upon cleavage. The prodrug, for example, can
10 contain an oligopeptide, typically a relatively short, less than about 10 amino acids peptide, that is proteolytically cleaved by the targeted CVSP14. Cytotoxic agents, include, but are not limited to, alkylating agents, antiproliferative agents and tubulin binding agents. Others include, vinca drugs, mitomycins, bleomycins and taxanes.

15 M. Animal models

Transgenic animal models and animals, such as rodents, including mice
an rats, cows, chickens, pigs, goats, sheep, gorillas and other primates, are provided herein. In particular, transgenic non-human animals that contain heterologous nucleic acid encoding a CVSP14 polypeptide or a transgenic animal
20 in which expression of the polypeptide has been altered, such as by replacing or modifying the promoter region or other regulatory region of the endogenous gene are provided.

Such an animal can be produced by promoting recombination between an exogenous CVSP14 gene that could be over-expressed or mis-expressed, such
25 as by expression under a strong promoter, via homologous or other recombination event. For example, transgenic animals can be produced by introducing the nucleic acid using vectors or other modes of gene delivery into a germline cell, such as an embryonic stem cell. Typically the nucleic acid is introduced, such as an embryonic stem cell, which is then injected by transforming embryo-
30 derived stem (ES) cells with a vector containing the CVSP14 polypeptide-encoding nucleic acid followed by injecting the ES cells into a blastocyst, and implanting the blastocyst into a foster mother, followed by the birth of a

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transgenic animal. Generally introduction into a chromosome of the animal occurs by a recombination between the heterologous CVSP14-encoding nucleic acid and endogenous nucleic acid. The heterologous nucleic acid can be targeted to a specific chromosome. In some instances, knockout animals can be produced. Such an animal can be initially produced by promoting homologous recombination between a CVSP14 polypeptide gene in its chromosome and an exogenous CVSP14 polypeptide gene that has been rendered biologically inactive (typically by insertion of a heterologous sequence, *e.g.*, an antibiotic resistance gene). In one embodiment, this homologous recombination is performed by transforming embryo-derived stem (ES) cells with a vector containing the insertionally inactivated CVSP14 polypeptide gene, such that homologous recombination occurs, followed by injecting the ES cells into a blastocyst, and implanting the blastocyst into a foster mother, followed by the birth of the chimeric animal ("knockout animal") in which a CVSP14 polypeptide gene has been inactivated (see Capecchi, *Science* 244:1288-1292 (1989)). The chimeric animal can be bred to produce homozygous knockout animals, which can then be used to produce additional knockout animals.

Knockout animals include, but are not limited to, mice, hamsters, sheep, pigs, cattle, and other non-human mammals. For example, a knockout mouse is produced. Such knockout animals are expected to develop or be predisposed to developing neoplastic diseases and thus can have use as animal models of such diseases *e.g.*, to screen for or test molecules for the ability to treat or prevent such diseases or disorders. Such an animal can be initially produced by promoting homologous recombination between a CVSP14 gene in its chromosome and an exogenous CVSP14 polypeptide gene that would be over-expressed or mis-expressed (generally by expression under a strong promoter). In an embodiment, this homologous recombination is carried out by transforming embryo-derived stem (ES) cells with a vector containing the over-expressed or mis-expressed CVSP14 polypeptide gene, such that homologous recombination occurs, followed by injecting the ES cells into a blastocyst, and implanting the blastocyst into a foster mother, followed by the birth of the chimeric animal in which a CVSP14 gene has been over-expressed or mis-expressed (see Capecchi,

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Science 244:1288-1292 (1989)). The chimeric animal can be bred to produce additional animals with over-expressed or mis-expressed CVSP14 polypeptide. Such animals include, but are not limited to, mice, hamsters, sheep, pigs, cattle and other non-human mammals. In a specific embodiment, a mouse with over-expressed or mis-expressed CVSP14 polypeptide is produced.

The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLE 1

10 Identification of CVSP14

Preparation of Single Strand cDNA from Prostate Tumor Samples

The human prostate tumor CWR22R was grown on nude mice. CWR22R tissue was dissected and put into TRIZOL Reagent (Gibco BRL) and total RNA was purified according to the manufacturer's instructions. Poly A⁺ RNA was further purified from total RNA using Oligotex mRNA mini Kit (Qiagen). Single strand cDNA was synthesized using SuperScript First-Strand Synthesis System (Gibco BRL). Either random hexamers or oligo(dT) was used to prime the first-strand cDNA synthesis.

Serine Protease Profiling by Degenerate Primer PCR

20 Serine protease domains were amplified using degenerate primers designed from the consensus sequences flanking the catalytic histidine (DSPP1) and the catalytic serine (DSPP2). The sequence of the sense primer (DSPP1) used is as follows (SEQ ID No. 7) : 5'-TGG (GA)TI (ACG)TI (TA)(CG)I GCI (AG)CI CA(TC) TG-3' (nucleotides in parentheses represent equal molar mixtures and I represents deoxyinosine). The sequence of antisense primer (DSPP2) used is as follows (SEQ ID No. 8): 5'-IGG ICC ICC I(CG)(TA) (GA)TC ICC (TC)TI (AG)CA IG(TAC) (AG)TC-3'. Random hexamer and oligo(dT) primed cDNA were used as templates for PCR reactions. PCR products were separated on agarose gels, and all products between 450- to 550-bp were extracted from the gels and subcloned into the pCR2.1-TOPO cloning vector (Invitrogen). Plasmids containing PCR-generated inserts were identified by electrophoresis of EcoR I digestion products on agarose gels. Plasmids containing 450-550 bp inserts

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were subjected to DNA sequencing. One of these clones contained a 474 bp insert that encoded a portion of the protease domain of a novel serine protease. This serine protease sequence is hereafter referred to as CVSP14.

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5 PCR reactions. PCR products were separated on agarose gels, and all products between 450- to 550-bp were extracted from the gels and subcloned into the pCR2.1-TOPO cloning vector (Invitrogen). Plasmids containing PCR-generated inserts were identified by electrophoresis of EcoR I digestion products on agarose gels. Plasmids containing 450-550 bp inserts were subjected to DNA
10 sequencing. One of these clones contained a 474 bp insert that encoded a portion of the protease domain of a serine protease, which is referred to as CVSP14 herein.

A BLAST search against the human genomic database *htgs* (Unfinished High Throughput Genomic Sequences) revealed that this sequence matches a
15 genomic sequence AC012228 that is derived from human chromosome 11.

Cloning of cDNA Encoding the Protease Domain of CVSP14.

ClonCapture cDNA Selection Kit (Clontech) was used to obtain cDNA encoding the CVSP14 protease domain. A biotinylated 474 bp partial cDNA clone for CVSP14 was generated by PCR using DSPP1 and DSPP2 primers in the
20 presence of biotin-21-dUTP. The biotinylated product was gel purified and used as probe in RecA-mediated ClonCapture procedures. Human prostate adenocarcinoma cDNA library (Gibco BRL Cat. # 11597-010) was used as the cDNA source. The captured cDNAs were transformed into ElectroMAX DH10B cells by electroporation, and positive clones containing CVSP14 protease domain
25 were identified by colony hybridization using a non-biotinylated DSPP1 and DSPP2 PCR product. Positive clones were verified by DNA sequencing. DNA sequencing analysis of four positive clones indicated that all clones contained cDNAs encoding the protease domain of a serine protease. The cDNA encoding CVSP14 protease domain is composed of 756 bp, which translates
30 into 251-amino acids. BLAST analysis of the protein database indicated that this serine protease has highest homology to one of the serine protease domains of

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Xenopus oviductin (Genbank accession number U81291 and T30338) with 47% identity.

Cloning of human CVSP14 full-length cDNA

To obtain the remaining 5' upstream cDNA of CVSP14, 5'-RACE

- 5 reactions were performed on the human kidney RACE cDNA synthesized using GeneRacer Kit (Ambion, Cat. No. L1500-01). GeneRacer kit is specifically designed for full-length, RNA ligase-mediated rapid amplification of 5' and 3' cDNA ends (RLM-RACE). The first 5'-RACE reaction was performed by PCR using GeneRacer 5' primer with gene specific primers, GX-SP1-4AS, 5'-
- 10 GTTAAGCGGCCCCAGCCTGCAGTTGTAC-3' SEQ ID NO. . The PCR products were purified from agarose gel.

A second nested PCR was then performed using GeneRacer 5' nested primer with gene specific primer GX-SP1-1AS, 5'-

GCTCTCCTGGGTCTGTCTGGCTTAAGTC-3' SEQ ID NO. 19 (using first 5'-

- 15 RACE product as template). The PCR products from RACE reactions, which were greater than 500 bp, were purified from agarose gel and subcloned into pCR2.1-TOPO cloning vector (Invitrogen, Carlsbad, CA). Colony hybridization was then performed to identify positive colonies containing CVSP14 sequence. An additional sequence of 279 bp was obtained from the second 5'-RACE
- 20 products including an ATG start codon within a sequence of AAAACTATGAGT (SEQ ID NO. 20).

Nucleotide and protein Sequence of the CVSP14

The nucleotide and Amino Acid sequences of Human CVSP14 are set forth below and in SEQ ID Nos. 12 and 13:

- 25 GAT TCA CCA CGT CTT GGT TAA TGA ATA AAC TTG TTT TAA ATT GGC TTA TTG CTG
- GTC TCT CAA GGC TTC CTA TTT TTG TTT GCT TTA GTC TCT CTA AAA TTT CAG GGA AAA ACT
115/1 145/11
- 30 ATG AGT CTC AAA ATG CTT ATA AGC AGG AAC AAG CTG ATT TTA CTA CTA GGA ATA GTC TTT
M S L K M L I S R N K L I L L L G I V F
-
- 175/21 205/31
- TTT GAA CAA GGT AAA TCT GCA GCT CTT TCG CTC CCC AAA GCT CCC AGT TGT GGG CAG AGT
F E Q G K S A A L S L P K A P S C G Q S
- 235/41 265/51
- 35 CTG GTT AAG GTA CAG CCT TGG AAT TAT TTT AAC ATT TTC AGT CGC ATT CTT GGA GGA AGC
L V K V Q P W N Y F N I F S R I L G G S
- 295/61 325/71
- CAA GTG GAG AAG GGT TCC TAT CCC TGG CAG GTA TCT CTG AAA CAA AGG CAG AAG CAT ATT
Q V E K G S Y P W Q V S L K Q R Q K H I
- 40 355/81 385/91
- TGT GGA GGA AGC ATC GTC TCA CCA CAG TGG GTG ATC ACG GCG GCT CAC TGC ATT GCA AAC

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C   G   G   S   I   V   S   P   Q   W   V   I   T   A   A   H   C   I   A   N
415/101
AGA AAC ATT GTG TCT ACT TTG AAT GTT ACT GCT GGA GAG TAT GAC TTA AGC CAG ACA GAC
R   N   I   V   S   T   L   N   V   T   A   G   E   Y   D   L   S   Q   T   D
5   475/121
CCA GGA GAG CAA ACT CTC ACT ATT GAA ACT GTC ATC ATA CAT CCA CAT TTC TCC ACC AAG
P   G   E   Q   T   L   T   I   E   T   V   I   I   H   P   H   F   S   T   K
535/141
AAA CCA ATG GAC TAT GAT ATT GCC CTT TTG AAG ATG GCT GGA GCC TTC CAA TTT GGC CAC
10  K   P   M   D   Y   D   I   A   L   L   K   M   A   G   A   F   Q   F   G   H
595/161
TTT GTG GGG CCC ATA TGT CTT CCA GAG CTG CGG GAG CAA TTT GAG GCT GGT TTT ATT TGT
F   V   G   P   I   C   L   P   E   L   R   E   Q   F   E   A   G   F   I   C
655/181
ACA ACT GCA GGC TGG GGC CGC TTA ACT GAA GGT GGC GTC CTC TCA CAA GTC TTG CAG GAA
15  T   T   A   G   W   G   R   L   T   E   G   G   V   L   S   Q   V   L   Q   E
715/201
GTG AAT CTG CCT ATT TTG ACC TGG GAA GAG TGT GTG GCA GCT CTG TTA ACA CTA AAG AGG
V   N   L   P   I   L   T   W   E   E   C   V   A   A   L   L   T   L   K   R
20  775/221
CCC ATC AGT GGG AAG ACC TTT CTT TGC ACA GGT TTT CCT GAT GGA GGG AGA GAC GCA TGT
P   I   S   G   K   T   F   L   C   T   G   F   P   D   G   G   R   D   A   C
835/241
CAG GGA GAT TCA GGA GGT TCA CTC ATG TGC CGG AAT AAG AAA GGG GCC TGG ACT CTG GCT
25  Q   G   D   S   G   G   S   L   M   C   R   N   K   K   G   A   W   T   L   A
895/261
GGT GTG ACT TCC TGG GGT TTG GGC TGT GGT CGA GGC TGG AGA AAC AAT GTG AGG AAA AGT
G   V   T   S   W   G   L   G   C   G   R   G   W   R   N   N   V   R   K   S
955/281
GAT CAA GGA TCC CCT GGG ATC TTC ACA GAC ATT AGT AAA GTG CTT TCC TGG ATC CAC GAA
30  D   Q   G   S   P   G   I   F   T   D   I   S   K   V   L   S   W   I   H   E
1015/301
CAC ATC CAA ACT GGT AAC TAA
H   I   Q   T   G   N   *
35

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*Underline indicates the signal peptide

Sequence analysis and domain organization of CVSP14

The CVSP14 DNA and protein sequences were analyzed using DNA
 40 Strider (version 1.2). The ORF of CVSP14 is composed of 921 bp, which
 translate into a 306-amino acid protein. Protein sequence analysis using the
 SMART (Simple Modular Architecture Research Tool) program at
<http://smart.embl-heidelberg.de> indicates that CVSP14 is a secreted serine
 protease with a signal peptide (amino acids 1-25) at the N-terminus followed by
 45 a trypsin-like serine protease domain (amino acids 55-306).
 The amino acid and nucleotide sequences are set forth in SEQ ID No. 12 and 13.

Gene expression profile of CVSP14 in normal and tumor tissues

To obtain information regarding the gene expression profile of the
 CVSP14 transcript, PCR analysis was carried out on cDNA panels made from
 50 several human adult tissues (Clontech, Cat. #K1420-1), fetal tissues (Cat.
 #K1425-1) and primary tumors (human tumor multiple tissue cDNA panel,
 catalog number K1522-1, CLONTECH) using CVSP14-specific primers GX-SP1-1

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(SEQ ID No. 9) (5'-GACTTAAGCCAGACAGACCCAGGAGAGC-3') and
GX-SP1-2AS

(5'-TTGTGAGAGGACGCCACCTTCAGTTAAGC-3') (SEQ ID No. 10).

After 35 PCR cycles, a DNA band (246 bp) of strong intensity, indicating
5 high expression of CVSP14, was detected only in kidney cDNA. A DNA band of
moderate intensity was seen in lung cDNA, and a weak band was seen in
placenta cDNA. No detectable signal was observed in either fetal tissue or
tumor cDNA. After 40 PCR cycles, additional signals can be detected in adult
liver, pancreas, fetal heart, fetal lung, fetal skeletal muscle, fetal thymus, colon
10 adenocarcinoma (CX-1), and pancreatic adenocarcinoma (GI-103).

A PCR product of 474 bp generated by DSPP1 and DSPP2 primers was
used to probe a cDNA blot composed of cDNA synthesized from 68 human
tumors and corresponding normal tissue from the same individual (catalog
number 7840-1 human matched tumor/normal expression array; CLONTECH) as
15 well as a dot blot composed of RNA extracted from 72 different human tissues
(Human Multiple Tissue Expression (MTE) Array; Clontech; Palo Alto, CA;
catalog no. 7776-1). Strong signals, indicating high expression of CVSP14,
were detected in 6 of the 15 normal kidney cDNA samples and moderate to
weak signals could also be detected in 8 additional normal kidney cDNA
20 samples. CVSP14 signals were diminished in all the matched kidney tumor
samples. Weak signals were detected in all three pairs of prostate normal/tumor
cDNA samples. Weak signals were also detected in 3 of 9 normal breast
samples. A weak signal was also detected in one of the 7 uterine tumors, but
not in their normal tissue counterparts. Weak signals were also detected in two
25 of the three normal lung tissue samples, but not in their matched tumor samples.
Very weak signals can be seen in cDNA samples from various tumor cell lines,
including HeLa cells, Burkitt's lymphoma Daudi cells, chronic myelogenous
leukemia K562, promyelocytic leukemia HL-60 cells, melanoma G361 cells, lung
carcinoma A549 cells, lymphoblastic leukemia MOLT-4 and colorectal
30 adenocarcinoma SW480 cells.

The results of MTE analysis indicated that CVSP14 transcript is
expressed moderately in lymph node and weakly in heart, stomach, duodenum,

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jejunum, ileum, ileocecum, colon (ascending, transverse, and descending), kidney, skeletal muscle, lung, placenta, liver, pancreas and salivary gland.

EXAMPLE 2

Expression of the protease CVSP domains

5 Nucleic acid encoding each the CVSP14 and protease domain thereof can be cloned into a derivative of the *Pichia pastoris* vector pPIC9K (available from Invitrogen; see SEQ ID NO. 11). Plasmid pPIC9K features include the 5' AOX1 promoter fragment at 1-948; 5' AOX1 primer site at 855-875; alpha-factor secretion signal(s) at 949-1218; alpha-factor primer site at 1152-1172; multiple
10 cloning site at 1192-1241; 3' AOX1 primer site at 1327-1347; 3' AOX1 transcription termination region at 1253-1586; HIS4 ORF at 4514-1980; kanamycin resistance gene at 5743-4928; 3' AOX1 fragment at 6122-6879; ColE1 origin at 7961-7288; and the ampicillin resistance gene at 8966-8106. The plasmid is derived from pPIC9K by eliminating the XhoI site in the
15 kanamycin resistance gene and the resulting vector is herein designated pPIC9Kx.

C122S mutagenesis of the Protease domain of CVSP14

The gene encoding the protease domain of CVSP14 was mutagenized by PCR SOE (PCR-based splicing by overlap extension) to replace the unpaired
20 cysteine at position 122 (chymotrypsin numbering system; cysteine 166 in CVSP14 SEQ ID No. 13) with a serine. Two overlapping gene fragments, each containing the AGT codon for serine at position 166 were PCR amplified using the following primers: for the 5' gene fragment:
TCTCTCGAGAAAAGAATTCTTGGAGGAAGCCAAGTGGAG (SEQ ID No. 14) and
25 TTTGTGGGGCCCATAAGTCTTCCAGAGCTGCGG (SEQ ID No. 15); for the 3' gene fragment, ATTCGCGGCCGCTTAGTT-ACCAAGTTTGGATGTGTTTCGTG (SEQ ID No. 16) and CCGCAGCTCTGGAAGACTTATGGGCCCCACAAA (SEQ ID No. 17). The amplified gene fragments were purified on a 1% agarose gel, mixed and reamplified by PCR to produce the full length coding sequence for
30 for the protease domain of CVSP14 C122S (Cys₁₆₆ Seq ID No 13; Cys₁₁₁ Seq ID No. 6). This sequence was then cut with restriction enzymes NotI and XhoI, and ligated into vector pPic9KX.

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Construction of CVSP14 expression vector

cDNA encoding CVSP14 containing the C122S point mutation (i.e., CVSP14C122S, position C₁₆₆ in SEQ ID Nos. 12 and 13) was cloned from pPIC9Kx:CVSP14C122S. The primers CVSP14-5'

- 5 GGAATTCCATATGAGCAGCGGCCATATCGACGACGACGACAAAAATTCTTGAG
GAAGCCAAGTGGAG (containing a NdeI restriction site; SEQ ID No. 21) and
CVSP14-3' CCGCTCGAGGTTACCAGTTTGGATGTGTTTCGTGG (containing a
XhoI restriction site; SEQ ID No. 22) were used to PCR amplify the human
CVSP14 protease domain utilizing an enterokinase recognition sequence
10 (DDDDK) for zymogen activation. Amplification was conducted in a total
volume of 50ul containing 20mM tris-HCl (pH 8.75 at 25°C), 10mM KCl, 10
mM (NH₄)₂SO₄, 2mM MgSO₄, 0.1% triton X-100, 0.1mg/ml BSA, 0.2mM dNTPs,
1.0 unit ACCUZYME DNA polymerase (Bioline USA, Inc., New Jersey), and 100
15 pmol of primers. The reaction mixture was heated to 95°C for 5 min, followed
by 25 cycles of 95, 60, and 75°C for 30 s each and a final extension of 75°C
for 2 min.

- PCR products were purified using a QIAquick PCR purification kit
(QIAGEN Inc., Chatsworth, CA). PCR products were doubly digested with 10
units NdeI and 10 units XhoI for 2 hrs at 37°C. The digested fragments were
20 purified on a 1.4% agarose gel and stained with ethidium bromide. The band
containing CVSP14 cDNA was excised and purified using a QIAEX II gel
extraction kit (QIAGEN Inc., Chatsworth, CA). CVSP14 cDNA was then cloned
into the NdeI and XhoI sites of the pET21b expression vector (Novagen, Inc.,
Madison, WI) using standard methods. This vector allows the fusion of a C-
25 terminal 6xHIS tag for purification by immobilized metal affinity chromatography
(IMAC). Competent XL10 cells (Stratagene) were transformed with the
pET21bCVSP14 vector and used to produce plasmid stocks. Proper insertion
and DNA sequence were confirmed by fluorescent thermal dye DNA sequencing
methods as well as restriction digests.

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Protein Expression, Purification, and Refolding

Overexpression of the gene product was achieved in *E. coli* strain BL21(DE3) containing the dnaY plasmid for rare codon optimization (Garcia *et al.* (1986) *Cell* 45:453-459; see, U.S. Patent No. 6,270,988). 2xYT media (1L),
5 supplemented with carbanicillin (50ug/ml) and kanamycin (34ug/ml), was inoculated with a 10 ml overnight culture and grown to a density of 0.6-1.0 OD600 before induction with 1M IPTG (1mM final concentration). After 6 hours post-induction growth, cells were harvested by centrifugation (3000g x 20 minutes).

10 The cell pellet was resuspended in 50mM NaH₂PO₄, 300mM NaCl, 5% LADO, pH 7.4 (25 mL) supplemented with 5-10mg lysozyme and 1U DNaseI to lyse the cells and shear the DNA. The resulting solution was then centrifuged at 48,000g for 20 minutes. The supernatant was discarded and the inclusion body pellet was washed by homogenization with the lysis buffer followed by the lysis
15 buffer minus detergent with centrifugation as described above between washes. The inclusion body pellet was then dissolved in 25 mL 6M GuHCl, 20 mM tris-HCl, 300 mM NaCl, 20mM β Me, pH 8.0. This solution was then centrifuged at 48,000g for 30 minutes to remove particulate matter.

The resulting solution was filtered through a 0.2um syringe filter before
20 loading onto 25ml Ni-NTA resin (QIAGEN Inc., Chatsworth, CA) pre-equilibrated with 6M GuHCl, 20 mM tris-HCl, 300 mM NaCl, pH 8.0. The column was washed with two column volumes equilibration buffer followed by three column volumes 8M urea, 20 mM tris-HCl, 300 mM NaCl, pH 8.0. Purified inclusion
bodies are then eluted with two column volumes 8M urea, 20 mM tris-HCl, 300
25 mM NaCl, 1M imidazole, pH 8.0.

CVSP14 was refolded by slowly adding the inclusion body mixture to 8L 100mM tris-HCl, 150mM NaCl, 7.5mM cysteine, 1mM cystine, 0.5M arginine, 3g/L cholic acid, pH 8.0 using a peristaltic pump. The refolding mixture was allowed to stir at 4°C for 7 days or until thiol concentration was below 1mM as
30 detected by Ellman's reagent. The refolding solution was filtered through a 1uM filter, concentrated by ultrafiltration and the buffer exchanged in PBS, 3g/L cholic acid, pH 8.0.

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Activation of CVSP14 was performed by the addition of 1-10 U/ml EKMax (Invitrogen, Carlsbad, CA) and incubation at 4°C until the reaction was deemed complete (generally 4-8 days). Residual EKMax was removed by treating the solution with a small amount of ConA resin that binds the glycosylated enterokinase. Complete removal of EKMax was confirmed by measuring the activity of the solution towards a specific enterokinase fluorogenic substrate.

The resulting solution was screened for activity against a series of protease substrates; spec-tPa, spec-PL, spec-fXIIa (American Diagnostica), S-2239, S-2266 (Kabi Diagnostica), S-2586, S-2366, S-2444, S-2288, S-2251, S-2302, S-2765, S-2222, spec-TH (Chromogenix), and spec-fVIIa (Pentapharm). CVSP14 exhibited some activity towards a number of these substrates, but was most active towards S-2366 (DiaPharma, Westchester, OH).

EXAMPLE 3

Assays for identification of candidate compounds that modulate that activity of a CVSP

Assay for identifying inhibitors

The ability of test compounds to act as inhibitors of catalytic activity of a CVSP14 can be assessed in an amidolytic assay. The inhibitor-induced inhibition of amidolytic activity by a recombinant CVSP or the protease domain portions thereof, can be measured by IC₅₀ values in such an assay.

The protease domain of CVSP14 expressed as described above is assayed in Costar 96 well tissue culture plates (Corning NY) for inhibition by various test compounds as follows. Approximately 1-10 nM protease is added without inhibitor, or with 100000 nM inhibitor and seven 1:6 dilutions into 1X direct buffer (29.2 mM Tris, pH 8.4, 29.2 mM Imidazole, 217 mM NaCl (100 µL final volume)), and allowed to incubate at room temperature for 30 minutes. 400 µM substrate S 2366 (L-pyroglutamyl-L-prolyl-L-arginine-p-nitroaniline hydrochloride; DiaPharma, Westchester, OH) is added and the reaction is monitored in a SpectraMAX Plus microplate reader (Molecular Devices, Sunnyvale CA) by following change in absorbance at 405 nm for 20 minutes at 37°C.

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Identification of substrates

Particular substrates for use in the assays can be identified empirically by testing substrates. The following list of substrates are exemplary of those that can be tested.

5	Substrate name	Structure
	S 2366	pyroGlu-Pro-Arg-pNA.HCl
	spectrozyme t-PA	CH ₃ SO ₂ -D-HHT-Gly-Arg-pNA.AcOH
	N-p-tosyl-Gly-Pro-Arg-pNA	N-p-tosyl-Gly-Pro-Arg-pNA
	Benzoyl-Val-Gly-Arg-pNA	Benzoyl-Val-Gly-Arg-pNA
10	Pefachrome t-PA	CH ₃ SO ₂ -D-HHT-Gly-Arg-pNA
	S 2765	N- α -Z-D-Arg-Gly-Arg-pNA.2HCl
	S 2444	pyroGlu-Gly-Arg-pNA.HCl
	S 2288	H-D-Ile-Pro-Arg-pNA.2HCl
	spectrozyme UK	Cbo-L-(γ)Glu(α -t-BuO)-Gly-Arg-pNA.2AcOH
15	S 2302	H-D-Pro-Phe-Arg-pNA.2HCl
	S 2266	H-D-Val-Leu-Arg-pNA.2HCl
	S 2222	Bz-Ile-Glu(g-OR)-Gly-Arg-pNA.HCl R = H(50%) and R = CH ₃ (50%)
	Chromozyme PK	Benzoyl-Pro-Phe-Arg-pNA
	S 2238	H-D-Phe-Pip-Arg-pNA.2HCl
20	S 2251	H-D-Val-Leu-Lys-pNA.2HCl
	Spectrozyme PI	H-D-Nle-HHT-Lys-pNA.2AcOH
		Pyr-Arg-Thr-Lys-Arg-AMC
		H-Arg-Gln-Arg-Arg-AMC
		Boc-Gln-Gly-Arg-AMC
25		Z-Arg-Arg-AMC
	Spectrozyme THE	H-D-HHT-Ala-Arg-pNA.2AcOH
	Spectrozyme fXIIa	H-D-CHT-Gly-Arg-pNA.2AcOH
		CVS 2081-6 (MeSO ₂ -dPhe-Pro-Arg-pNA)
		Pefachrome fVIIa (CH ₃ SO ₂ -D-CHA-But-Arg-pNA)

30 pNA = para-nitranilide (chromogenic)
AMC = amino methyl coumarin (fluorescent)

If none of the above substrates are cleaved, a coupled assay, described above, can be used. Briefly, test the ability of the protease to activate and enzyme, such as plasminogen and trypsinogen. To perform these assays, the
35 single chain protease is incubated with a zymogen, such as plasminogen or trypsinogen, in the presence of the a known substrate, such, lys-plasminogen,



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for the zymogen. If the single chain activates the zymogen, the activated enzyme, such as plasmin and trypsin, will degrade the substrate therefor.

EXAMPLE 4

Other Assays

- 5 These assays are described with reference to MTSP1, but such assays can be readily adapted for use with CVSP14.

Amidolytic Assay for Determining Inhibition of Serine Protease Activity of Matriptase or MTSP1

- The ability of test compounds to act as inhibitors of rMAP catalytic
10 activity was assessed by determining the inhibitor-induced inhibition of amidolytic activity by the MAP, as measured by IC_{50} values. The assay buffer was HBSA (10 mM Hepes, 150mM sodium chloride, pH 7.4, 0.1% bovine serum albumin). All reagents were from Sigma Chemical Co. (St. Louis, MO), unless otherwise indicated.
- 15 Two IC_{50} assays (a) one at either 30-minutes or 60-minutes (a 30-minute or a 60-minute preincubation of test compound and enzyme) and (b) one at 0-minutes (no preincubation of test compound and enzyme) were conducted. For the IC_{50} assay at either 30-minutes or 60-minutes, the following reagents
20 were combined in appropriate wells of a Corning microtiter plate: 50 microliters of HBSA, 50 microliters of the test compound, diluted (covering a broad concentration range) in HBSA (or HBSA alone for uninhibited velocity measurement), and 50 microliters of the rMAP (Corvas International) diluted in buffer, yielding a final enzyme concentration of 250 pM as determined by active site filtration. Following either a 30-minute or a 60-minute incubation at ambient
25 temperature, the assay was initiated by the addition of 50 microliters of the substrate S-2765 (N- α -Benzyloxycarbonyl-D-arginyl-L-glycyl-L-arginine-p-nitroaniline dihydrochloride; DiaPharma Group, Inc.; Franklin, OH) to each well, yielding a final assay volume of 200 microliters and a final substrate concentration of 100 μ M (about 4-times K_m). Before addition to the assay
30 mixture, S-2765 was reconstituted in deionized water and diluted in HBSA. For the IC_{50} assay at 0 minutes; the same reagents were combined: 50 microliters of HBSA, 50 microliters of the test compound, diluted (covering the identical

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concentration range) in HBSA (or HBSA alone for uninhibited velocity measurement), and 50 microliters of the substrate S-2765. The assay was initiated by the addition of 50 microliters of rMAP. The final concentrations of all components were identical in both IC_{50} assays (at 30- or 60- and 0-minute).

5 The initial velocity of chromogenic substrate hydrolysis was measured in both assays by the change of absorbance at 405 nM using a Thermo Max[®] Kinetic Microplate Reader (Molecular Devices) over a 5 minute period, in which less than 5% of the added substrate was used. The concentration of added inhibitor, which caused a 50% decrease in the initial rate of hydrolysis was
10 defined as the respective IC_{50} value in each of the two assays (30- or 60-minutes and 0-minute).

***In vitro* enzyme assays for specificity determination**

The ability of compounds to act as a selective inhibitor of matriptase activity was assessed by determining the concentration of test compound that
15 inhibits the activity of matriptase by 50%, (IC_{50}) as described in the above Example, and comparing IC_{50} value for matriptase to that determined for all or some of the following serine proteases: thrombin, recombinant tissue plasminogen activator (rt-PA), plasmin, activated protein C, chymotrypsin, factor
20 Xa and trypsin.

20 The buffer used for all assays was HBSA (10 mM HEPES, pH 7.5, 150 mM sodium chloride, 0.1% bovine serum albumin).

The assay for IC_{50} determinations was conducted by combining in appropriate wells of a Corning microtiter plate, 50 microliters of HBSA, 50 microliters of the test compound at a specified concentration (covering a broad
25 concentration range) diluted in HBSA (or HBSA alone for V_0 (uninhibited velocity) measurement), and 50 microliters of the enzyme diluted in HBSA. Following a 30 minute incubation at ambient temperature, 50 microliters of the substrate at the concentrations specified below were added to the wells, yielding a final total volume of 200 microliters. The initial velocity of chromogenic substrate
30 hydrolysis was measured by the change in absorbance at 405 nm using a Thermo Max[®] Kinetic Microplate Reader over a 5 minute period in which less than 5% of the added substrate was used. The concentration of added inhibitor

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which caused a 50% decrease in the initial rate of hydrolysis was defined as the IC_{50} value.

Thrombin (fIIa) Assay

Enzyme activity was determined using the chromogenic substrate,

5 Pefachrome t-PA (CH_3SO_2 -D-hexahydrotyrosine-glycyl-L-Arginine-p-nitroaniline, obtained from Pentapharm Ltd.). The substrate was reconstituted in deionized water prior to use. Purified human α -thrombin was obtained from Enzyme Research Laboratories, Inc. The buffer used for all assays was HBSA (10 mM HEPES, pH 7.5, 150 mM sodium chloride, 0.1% bovine serum albumin).

10 IC_{50} determinations were conducted where HBSA (50 μ L), α -thrombin (50 μ l) (the final enzyme concentration is 0.5 nM) and inhibitor (50 μ l) (covering a broad concentration range), were combined in appropriate wells and incubated for 30 minutes at room temperature prior to the addition of substrate Pefachrome-t-PA (50 μ l) (the final substrate concentration is 250 μ M, about 5
15 times K_m). The initial velocity of Pefachrome t-PA hydrolysis was measured by the change in absorbance at 405 nm using a Thermo Max[®] Kinetic Microplate Reader over a 5 minute period in which less than 5% of the added substrate was used. The concentration of added inhibitor which caused a 50% decrease in the initial rate of hydrolysis was defined as the IC_{50} value.

20 Factor Xa

Factor Xa catalytic activity was determined using the chromogenic substrate S-2765 (N-benzyloxycarbonyl-D-arginine-L-glycine-L-arginine-p-nitroaniline), obtained from DiaPharma Group (Franklin, OH). All substrates were reconstituted in deionized water prior to use. The final concentration of S-2765
25 was 250 μ M (about 5-times K_m). Purified human Factor X was obtained from Enzyme Research Laboratories, Inc. (South Bend, IN) and Factor Xa (FXa) was activated and prepared from it as described [Bock, P.E., Craig, P.A., Olson, S.T., and Singh, P. *Arch. Biochem. Biophys.* 273:375-388 (1989)]. The enzyme was diluted into HBSA prior to assay in which the final concentration was 0.25 nM.

30 Recombinant tissue plasminogen activator (rt-PA) Assay

rt-PA catalytic activity was determined using the substrate, Pefachrome t-PA (CH_3SO_2 -D-hexahydrotyrosine-glycyl-L-arginine-p-nitroaniline, obtained from

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Pentapharm Ltd.). The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 500 micromolar (about 3-times K_m). Human rt-PA (Activase®) was obtained from Genentech Inc. The enzyme was reconstituted in deionized water and diluted
5 into HBSA prior to the assay in which the final concentration was 1.0 nM.

Plasmin Assay

Plasmin catalytic activity was determined using the chromogenic substrate, S-2366 (L-pyroglutamyl-L-prolyl-L-arginine-p-nitroaniline hydrochloride), which was obtained from DiaPharma group. The substrate was
10 made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 300 micromolar (about 2.5-times K_m). Purified human plasmin was obtained from Enzyme Research Laboratories, Inc. The enzyme was diluted into HBSA prior to assay in which the final concentration was 1.0 nM.

15 Activated Protein C (aPC) Assay

aPC catalytic activity was determined using the chromogenic substrate, Pefachrome PC (delta-carbobenzoxymethyl-L-lysine-L-prolyl-L-arginine-p-nitroaniline dihydrochloride), obtained from Pentapharm Ltd.). The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the
20 final concentration was 400 micromolar (about 3-times K_m). Purified human aPC was obtained from Hematologic Technologies, Inc. The enzyme was diluted into HBSA prior to assay in which the final concentration was 1.0 nM.

Chymotrypsin Assay

Chymotrypsin catalytic activity was determined using the chromogenic
25 substrate, S-2586 (methoxy-succinyl-L-arginine-L-prolyl-L-tyrosyl-p-nitroanilide), which was obtained from DiaPharma Group. The substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 100 micromolar (about 9-times K_m). Purified (3X-crystallized; CDI) bovine pancreatic alpha-chymotrypsin was obtained from Worthington
30 Biochemical Corp. The enzyme was reconstituted in deionized water and diluted into HBSA prior to assay in which the final concentration was 0.5 nM.

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Trypsin Assay

Trypsin catalytic activity was determined using the chromogenic substrate, S-2222 (benzoyl-L-isoleucine-L-glutamic acid-[gamma-methyl ester]-L-arginine-p-nitroanilide), which was obtained from DiaPharma Group. The

5 substrate was made up in deionized water followed by dilution in HBSA prior to the assay in which the final concentration was 250 micromolar (about 4-times K_m). Purified (3X-crystallized; TRL3) bovine pancreatic trypsin was obtained from Worthington Biochemical Corp. The enzyme was reconstituted in deionized water and diluted into HBSA prior to assay in which the final concentration was

10 0.5 nM.

Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

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WHAT IS CLAIMED IS:

1. A substantially purified single chain or two chain polypeptide, comprising the protease domain of serine protease 14 (CVSP14) or a catalytically active portion thereof or a domain thereof, wherein:

5 the polypeptide does not comprise the complete sequence set forth in SEQ ID No. 13; and

the CVSP14 portion of the polypeptide consists essentially of the protease domain of the CVSP14 or a catalytically active portion thereof.

2. A purified polypeptide of claim 1, comprising a sequence of amino
10 acids set forth in SEQ ID No. 6 or a catalytically active portion thereof.

3. A substantially purified activated two chain CVSP14 polypeptide or a catalytically active portion thereof.

4. The substantially purified activated two chain CVSP14 polypeptide of claim 3 that comprises a polypeptide selected from the group
15 consisting of:

a sequence of amino acids encoded by the sequence of nucleotides set forth in SEQ ID No. 5 or 12;

a polypeptide that comprises a sequence of amino acids encoded by the sequence of nucleotides set forth in SEQ ID No. 6 or 13;

20 a polypeptide that comprises a sequence of amino acids encoded by a sequence of nucleotides that hybridizes under conditions of high stringency to the sequence of nucleotides set forth in SEQ ID No. 5 or 12;

a polypeptide that comprises the sequence of amino acids set forth in SEQ ID No. 6 or 13;

25 a polypeptide that comprises a sequence of amino acids having at least about 50%, 60%, 70%, 80%, 90% or 95% sequence identity with the sequence of amino acids set forth in SEQ ID No. 6 or 13; and

a polypeptide that is encoded by a sequence of nucleotides that is a splice variant of the sequence set forth in SEQ ID No. 12.

30 5. A substantially purified polypeptide that has at least 50%, 60%, 70%, 80%, 90% or 95% sequence identity with the polypeptide of claim 1.

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6. The polypeptide of claim 1 that consists essentially of a protease domain or a catalytically active portion thereof.

7. A substantially purified polypeptide that has at least 50%, 60%, 70%, 80%, 90% or 95% sequence identity with the polypeptide of claim 1 and
5 retains at least 10% of the catalytic activity on the same substrate as the polypeptide of claim 1.

8. The substantially purified polypeptide of claim 1 that is a human polypeptide.

9. The polypeptide of claim 1 that comprises the sequence of amino
10 acids set forth in SEQ ID No. 6 or a catalytically active portion thereof, or that is encoded by a sequence of nucleotides that

(a) is set forth in SEQ ID No. 5;

(b) that hybridizes under conditions of moderate or high stringency to nucleic acid complementary to an mRNA transcript present in a
15 mammalian cell that encodes a CVSP14 encoded by (a);

(c) encodes a splice variant of (a); and

(d) comprises degenerate codons of the sequences of nucleotides of (a) or (b).

10. A substantially purified single chain or two chain polypeptide of
20 claim 1 that is encoded by a sequence of nucleotides comprising a sequence of nucleotides selected from the group consisting of:

(a) a sequence of nucleotides that encodes the protease domain that comprises the sequence of nucleotides set forth in SEQ ID Nos. 5 or 12;

25 (b) a sequence of nucleotides that hybridizes under conditions of moderate or high stringency to nucleic acid complementary to an mRNA transcript present in a mammalian cell that encodes a CVSP14 encoded by (a);

30 (c) a sequence of nucleotides that encodes a splice variant of (a) or (b); and

(d) a sequence of nucleotides that comprises degenerate codons of the sequences of nucleotides of (a) or (b).

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11. A substantially pure polypeptide of claim 1, wherein the protease domain portion is encoded by a nucleic acid molecule that hybridizes under conditions of high stringency along at least about 70% of the full length to a nucleic acid molecule comprising a sequence of nucleotides set forth in SEQ ID No: 5.

12. A polypeptide of claim 1, wherein the protease domain portion is encoded by a nucleic acid molecule that hybridizes under conditions of high stringency along at least 70% of its full length to a nucleic acid molecule comprising a sequence of nucleotides set forth in SEQ ID No: 15 or at least one domain thereof or a catalytically active portion of the domain.

13. A polypeptide that is a mutein of the polypeptide of claim 1, wherein:

up to about 50% of the amino acids are replaced with another amino acid;

and the resulting polypeptide is a single chain or two chain polypeptide that has catalytic activity of at least 10% of the unmutated polypeptide.

14. The polypeptide of claim 13, wherein up to about 10% of the amino acids are replaced with another amino acid.

15. The polypeptide of claim 13, wherein the resulting polypeptide is a single chain or two chain polypeptide and has catalytic activity of at least 50% of the unmutated polypeptide.

16. The polypeptide of claim 13, wherein a free Cysteine in the protease domain is replaced with another amino acid.

17. The polypeptide of claim 13, wherein up to about 95% of the amino acids are conserved or are replaced by conservative amino acid substitutions.

18. The polypeptide of claim 13, wherein the replacing amino acid is a serine.

19. A polypeptide that is a mutein of the polypeptide of claim 3, wherein:

up to about 50% of the amino acids are replaced with another amino acid;

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and the resulting polypeptide is a single chain or two chain polypeptide and has catalytic activity at least 10% of the unmutated polypeptide.

20. The polypeptide of claim 19, wherein up to about 10% of the amino acids are replaced with another amino acid;

5 21. The polypeptide of claim 19, wherein the resulting polypeptide is a two-chain polypeptide and has catalytic activity at least 50% of the unmutated polypeptide.

22. The polypeptide of claim 19, wherein a free Cys in the protease domain is replaced with another amino acid, whereby the resulting polypeptide
10 exhibits proteolytic activity.

23. The polypeptide of claim 22, wherein a free Cys in the protease domain is replaced with a serine.

24. A nucleic acid molecule, comprising a sequence of nucleotides that encodes the polypeptide of any of claims claim 1-23.

15 25. A nucleic acid molecule, comprising a sequence of nucleotides that encodes the polypeptide of claim 3.

26. A nucleic acid molecule, comprising a sequence of nucleotides that encodes the polypeptide of claim 6.

27. The polypeptide of claim 3 that consists essentially of the protease
20 domain.

28. The nucleic acid molecule of claim 24 that comprises a sequence of nucleotides selected from the group consisting of:

(a) a sequence of nucleotides set forth in SEQ ID No. 5 or 12;

(b) a sequence of nucleotides that hybridizes under high stringency along
25 at least about 70% of its full length to the sequence of nucleotides set forth in SEQ ID No. 5 or 12;

(c) degenerate codons of (a) or (b).

29. An isolated nucleic molecule that encodes a polypeptide of claim
13.

30 30. A vector comprising the nucleic acid molecule of claim 24.

31. The vector of claim 30 that is an expression vector.

32. The vector of claim 30 that is a eukaryotic vector.

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33. The vector of claim 31 that includes a sequence of nucleotides that directs secretion of any polypeptide encoded by a sequence of nucleotides operatively linked thereto.

34. The vector of claim 30 that is a *Pichia* vector, a mammalian vector
5 or an *E. coli* vector.

35. A cell, comprising the vector of claim 30.

36. The cell of claim 35 that is a prokaryotic cell.

37. The cell of claim 35 that is a eukaryotic cell.

38. The cell of claim 35 that is selected from among a bacterial cell, a
10 yeast cell, a plant cell, an insect cell and an animal cell.

39. The cell of claim 35 that is a mammalian cell.

40. A nucleic acid molecule encoding a polypeptide of claim 6.

41. A vector, comprising nucleic acid molecule of claim 40.

42. A cell, comprising the vector of claim 41.

15 43. A recombinant non-human animal, wherein an endogenous gene that encodes a polypeptide of claim 3 has been deleted or inactivated by homologous recombination or insertional mutagenesis of the animal or an ancestor thereof.

44. A method for producing a polypeptide that contains a protease
20 domain of a CVSP14 polypeptide, comprising:

culturing the cell of claim 35 under conditions whereby the encoded polypeptide is expressed by the cell; and
recovering the expressed polypeptide.

45. The method of claim 44, wherein the polypeptide is secreted into
25 the culture medium.

46. The method of claim 44, wherein the polypeptide is expressed in the cytoplasm of the host cell.

47. A method for producing a polypeptide that contains a protease domain of a polypeptide, comprising:

30 culturing the cell of claim 42 under conditions whereby the encoded polypeptide is expressed by the cell; and
recovering the expressed polypeptide.

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48. The method of claim 47, wherein the polypeptide is expressed in inclusion bodies, and the method further comprises

isolating the polypeptide from the inclusion bodies under conditions, whereby the polypeptide refolds into a proteolytically active form.

5 49. An antisense nucleic acid molecule that comprises at least 14 contiguous nucleotides or modified nucleotides that are complementary to a contiguous sequence of nucleotides encoding the protease domain of a CVSP14 of claim 1; or

 comprises at least 16 contiguous nucleotides or modified nucleotides that
10 are complementary to a contiguous sequence of nucleotides encoding the protease domain of a CVSP14 of claim 1; or

 comprises at least 30 contiguous nucleotides or modified nucleotides that are complementary to a contiguous sequence of nucleotides encoding the protease domain of a CVSP14 of claim 1.

15 50. The antisense molecule of claim 49 that includes a contiguous sequence of nucleotides set forth in SEQ ID No. 5 or 12.

 51. A double-stranded RNA (dsRNA) molecule that comprises at least about 21 contiguous nucleotides or modified nucleotides from the sequence of nucleotides encoding the CVSP14 of claim 1.

20 52. An antibody that specifically binds to the single chain form and/or two-chain form of a protease domain of the polypeptide of claim 1, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

 53. The antibody of claim 52 that inhibits the enzymatic activity of the
25 polypeptide.

 54. An antibody that specifically binds to the single chain form of a protease domain of the polypeptide of claim 1, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

30 55. An antibody that specifically binds to the polypeptide of claim 3 or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

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56. A conjugate, comprising:
- a) a polypeptide of claim 1, and
 - b) a targeting agent linked to the polypeptide directly or via a linker.
- 5 57. The conjugate of claim 56, wherein the targeting agent permits
- i) affinity isolation or purification of the conjugate;
 - ii) attachment of the conjugate to a surface;
 - iii) detection of the conjugate; or
 - iv) targeted delivery to a selected tissue or cell.
- 10 58. A conjugate, comprising:
- a) a polypeptide of claim 3; and
 - b) a targeting agent linked to the polypeptide directly or via a linker.
59. The conjugate of claim 58, wherein the targeting agent permits
- 15
- i) affinity isolation or purification of the conjugate;
 - ii) attachment of the conjugate to a surface;
 - iii) detection of the conjugate; or
 - iv) targeted delivery to a selected tissue or cell.
60. A conjugate, comprising:
- 20
- a) a polypeptide of claim 6; and
 - b) a targeting agent linked to the polypeptide directly or via a linker.
61. The conjugate of claim 60, wherein the targeting agent permits
- 25
- i) affinity isolation or purification of the conjugate;
 - ii) attachment of the conjugate to a surface;
 - iii) detection of the conjugate; or
 - iv) targeted delivery to a selected tissue or cell.
62. A combination, comprising:
- 30
- a) an agent or treatment that inhibits the catalytic activity of the polypeptide of claim 1; and
 - b) another treatment or agent selected from anti-tumor and anti-angiogenic treatments and agents.

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63. The combination of claim 62, wherein the inhibitor and the anti-tumor and/or anti-angiogenic agent are formulated in a single pharmaceutical composition or each is formulated in separate pharmaceutical compositions.

64. The combination of claim 62, wherein the inhibitor is selected from
5 antibodies and antisense oligonucleotides and double-stranded RNA (dsRNA).

65. A solid support comprising two or more polypeptides of claim 1 linked thereto either directly or via a linker.

66. The support of claim 65, wherein the polypeptides comprise an array.

10 67. The support of claim 65, wherein the polypeptides comprise a plurality of different protease domains.

68. A solid support comprising two or more nucleic acid molecules of claim 24 or oligonucleotides portions thereof linked thereto either directly or via a linker, wherein the oligonucleotides contain at least 16 nucleotides.

15 69. The support of claim 68, wherein the nucleic acid molecules comprise an array.

70. The support of claim 68, wherein the nucleic acid molecules comprise a plurality of molecules that encode different protease domains.

20 71. A method for identifying compounds that modulate the protease activity of a CVSP14 polypeptide, comprising:

contacting a CVSP14 polypeptide or a catalytically active portion thereof with a substrate that is proteolytically cleaved by the polypeptide, and, either simultaneously, before or after, adding a test compound or plurality thereof;
measuring the amount of substrate cleaved in the presence of the test
25 compound; and

selecting compounds that change the amount of substrate cleaved compared to a control, whereby compounds that modulate the activity of the polypeptide are identified.

72. The method of claim 71, wherein the test compounds are small
30 molecules, peptides, peptidomimetics, natural products, antibodies or fragments thereof that modulate the activity of the polypeptide.

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73. The method of claim 71, wherein a plurality of the test substances are screened simultaneously.

74. The method of claim 71, wherein the polypeptide consists essentially of a polypeptide encoded by a sequence of nucleotides selected from
5 the group consisting of a sequence of nucleotides that:

(a) is set forth in SEQ ID No. 5;

(b) hybridizes under conditions of high stringency to nucleic acid complementary to an mRNA transcript present in a mammalian cell that encodes CVSP14 encoded by (a);

10 (c) encodes a splice variant of (a) or (b); and

(d) comprises degenerate codons of the sequences of nucleotides of (a), (b) or (c).

75. The method of claim 71, wherein the polypeptide consists essentially of a polypeptide selected from the group consisting of:

15 a polypeptide that comprises a sequence of amino acids encoded by the sequence of nucleotides set forth in SEQ ID No. 5;

a polypeptide that comprises a sequence of amino acids encoded by the sequence of nucleotides set forth in SEQ ID No. 12;

20 a polypeptide that comprises a sequence of amino acids encoded by a sequence of nucleotides that hybridizes under conditions of high stringency to the sequence of nucleotides set forth in SEQ ID No. 5 or 12;

a polypeptide that comprises the sequence of amino acids set forth as amino acids of SEQ ID No. 6;

25 a polypeptide that comprises a sequence of amino acids having at least about 50%, 60%, 70%, 80%, 90% or 95% sequence identity with the sequence of amino acids set forth in SEQ ID No. 6 or 13; and

a polypeptide that is encoded by a sequence of nucleotides that is a splice variant of the sequence set forth in SEQ ID No. 13.

76. The method of claim 71, wherein the change in the amount of
30 substrate cleaved is assessed by comparing the amount of substrate cleaved in the presence of the test compound with the amount of substrate cleaved in the absence of the test compound.

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77. The method of claim 73, wherein a plurality of the polypeptides are linked to a solid support, either directly or via a linker.

78. The method of claim 73, wherein the polypeptides comprise an array.

5 79. A method of identifying a compound that specifically binds to a single-chain and/or two-chain protease domain and/or to single or two-chain polypeptide and/or to proteolytically active portion of the single or two chain form thereof of a CVSP14 polypeptide, comprising:

10 contacting a CVSP14 polypeptide or a proteolytically active portion thereof with a test compound or plurality thereof under conditions conducive to binding thereof; and either:

15 a) identifying test compounds that specifically bind to the single chain or two chain form of the polypeptide or to the single or to a two chain form thereof or to a proteolytically active portion of the single or two chain form thereof, or

b) identifying test compounds that inhibit binding of a compound known to bind a single chain or two chain form of the polypeptide or to the single or a two chain form thereof or to a proteolytically active portion of the single or two chain form thereof, wherein the known compound is contacted
20 with the polypeptide before, simultaneously with or after the test compound.

80. The method of claim 79, wherein the polypeptide is linked either directly or indirectly via a linker to a solid support.

81. The method of claim 79, wherein the test compounds are small molecules, peptides, peptidomimetics, natural products, antibodies or fragments
25 thereof.

82. The method of claim 79, wherein a plurality of the test substances are screened simultaneously.

83. The method of claim 79, wherein a plurality of the polypeptides are linked to a solid support.

30 84. The method of claim 79, wherein the polypeptide consists essentially of a polypeptide encoded by a sequence of nucleotides that:

(a) is set forth in SEQ ID No. 5;

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(b) hybridizes under conditions of moderate or high stringency to nucleic acid complementary to an mRNA transcript present in a mammalian cell that encodes a CVSP14 encoded by (a);

(c) encodes a splice variant of (a) or (b); and

5 (d) comprises degenerate codons of the sequences of nucleotides of (a), (b) or (c).

85. A method for identifying activators of the zymogen form of a CVSP14, comprising:

10 contacting a zymogen form of a CVSP14 polypeptide or a proteolytically active portion thereof with a substrate of the activated form of the polypeptide;

adding a test compound, wherein the test compound is added before, after or simultaneously with the addition of the substrate; and

15 detecting cleavage of the substrate, thereby identifying compounds that activate the zymogen.

86. The method of claim 85, wherein the substrate is a chromogenic substrate.

87. The method of claim 86, wherein the substrate is a L-pyroglutamyl-L-prolyl-L-arginine-p-nitroaniline hydrochloride.

20 88. The method of claim 85, wherein the test compound is a small molecule, a nucleic acid or a polypeptide.

89. The method of claim 85, wherein the polypeptide does not comprise the complete sequence set forth in SEQ ID No. 13; and

25 the CVSP14 portion of the polypeptide consists essentially of the protease domain of the CVSP14 or a catalytically active portion thereof.

90. A method for treating or preventing a neoplastic disease, in a mammal, comprising administering to a mammal an effective amount of an inhibitor of the proteolytic activity of a polypeptide of claim 1.

30 91. The method of claim 90, wherein the inhibitor is an antibody that specifically binds to the polypeptide, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

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92. A method for treating or preventing a neoplastic disease, in a mammal, comprising administering to a mammal an effective amount of an inhibitor of a polypeptide of claim 3.

93. A method of inhibiting tumor initiation, growth or progression or
5 treating a malignant or pre-malignant condition, comprising administering an agent that inhibits activation of the zymogen form of a CVSP14 polypeptide or a proteolytically active portion thereof or inhibits an activity of the activated form of CVSP14 or a proteolytically active portion thereof.

94. The method of claim 93, wherein the condition is a condition of
10 the breast, cervix, prostate, lung, ovary or colon.

95. The method of claim 93, wherein the agent is an antisense oligonucleotide, double-stranded RNA (dsRNA) or an antibody.

96. The method of claim 93, further comprising administering another
15 treatment or agent selected from anti-tumor and anti-angiogenic treatments or agents.

97. A method of identifying a compound that binds to the single-chain or two-chain form of a CVSP14 polypeptide or to a proteolytically active portion of a single-chain or two-chain form of a CVSP14 polypeptide, comprising:

contacting a test compound with both forms;

20 determining to which form the compound binds; and

if it binds to a form of polypeptide, further determining whether the compound has at least one of the following properties:

(i) inhibits activation of the single-chain zymogen form of polypeptide;

25 (ii) inhibits activity of the two-chain or single-chain form; and

(iii) inhibits dimerization of the polypeptide.

98. A method of detecting neoplastic disease, comprising: detecting a polypeptide that comprises a polypeptide of claim 1 in a biological sample, wherein the amount detected differs from the amount of polypeptide detected
30 from a subject who does not have neoplastic disease.

99. The method of claim 98, wherein the biological sample is selected from the group consisting of blood, urine, saliva, tears, synovial fluid, sweat,

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interstitial fluid, cerebrospinal fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells.

100. A method of diagnosing the presence of a pre-malignant lesion, a malignancy, or other pathologic condition in a subject, comprising:

- 5 obtaining a biological sample from the subject; and
 exposing it to a detectable agent that binds to a two-chain and/or single-chain form of a CVSP14 polypeptide, wherein the pathological condition is characterized by the presence or absence of the two-chain or single-chain form.

101. A method of monitoring tumor progression and/or therapeutic
10 effectiveness, comprising detecting and/or quantifying the level of a polypeptide of CVSP14 in a body tissue or fluid sample.

102. The method of claim 101, wherein the tumor is a tumor of the breast, cervix, prostate, lung, ovary or colon.

103. The method of claim 101, wherein the body fluid is blood, urine,
15 sweat, saliva, cerebrospinal fluid and synovial fluid.

104. A method for identifying compounds that modulate the protease activity of a CVSP14 polypeptide, comprising:

- contacting a polypeptide of claim 1 or a proteolytically active portion thereof with a substrate that is proteolytically cleaved by the polypeptide, and,
20 either simultaneously, before or after, adding a test compound or plurality thereof;

 measuring the amount of substrate cleaved in the presence of the test compound; and

 selecting compounds that change the amount of substrate cleaved
25 compared to a control, whereby compounds that modulate the activity of the polypeptide are identified.

105. A transgenic non-human animal, comprising heterologous nucleic acid encoding a polypeptide of claim 3.

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106. A polypeptide comprising a portion of a CVSP14 polypeptide, wherein the CVSP14 portion of the polypeptide consists essentially of amino acids 1-25 of SEQ ID No. 13.

107. A nucleic acid molecule encoding a polypeptide of claim 106.

-1-

SEQUENCE LISTING

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 Madison, Edwin
 Jiunn-Chern Yeh

<120> NUCLEIC ACID MOLECULES ENCODING SERINE PROTEASE CVSP14, THE ENCODED
 POLYPEPTIDES AND METHODS BASED THEREON

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-2-

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Asn	Ser	Asn	Ser	Thr	Glu	Phe	Val	Ser	Leu	Ala	Ser	Lys	Val	Lys	Asp	
			110					115					120			
gcg	ctg	aag	ctg	ctg	tac	agc	gga	gtc	cca	ttc	ctg	ggc	ccc	tac	cac	436
Ala	Leu	Lys	Leu	Leu	Tyr	Ser	Gly	Val	Pro	Phe	Leu	Gly	Pro	Tyr	His	
	125						130					135				
aag	gag	tgg	gct	gtg	acg	gcc	ttc	agc	gag	ggc	agc	gtc	atc	gcc	tac	484
Lys	Glu	Ser	Ala	Val	Thr	Ala	Phe	Ser	Glu	Gly	Ser	Val	Ile	Ala	Tyr	
	140					145					150					
tac	tgg	tct	gag	ttc	agc	atc	ccg	cag	cac	ctg	gtg	gag	gag	gcc	gag	532
Tyr	Trp	Ser	Glu	Phe	Ser	Ile	Pro	Gln	His	Leu	Val	Glu	Glu	Ala	Glu	
155					160					165					170	
cgc	gtc	atg	gcc	gag	gag	cgc	gta	gtc	atg	ctg	ccc	ccg	cgg	gcg	cgc	580
Arg	Val	Met	Ala	Glu	Glu	Arg	Val	Val	Met	Leu	Pro	Pro	Arg	Ala	Arg	
			175						180					185		
tcc	ctg	aag	tcc	ttt	gtg	gtc	acc	tca	gtg	gtg	gct	ttc	ccc	acg	gac	628
Ser	Leu	Lys	Ser	Phe	Val	Val	Thr	Ser	Val	Val	Ala	Phe	Pro	Thr	Asp	
			190					195					200			
tcc	aaa	aca	gta	cag	agg	acc	cag	gac	aac	agc	tgc	agc	ttt	ggc	ctg	676
Ser	Lys	Thr	Val	Gln	Arg	Thr	Gln	Asp	Asn	Ser	Cys	Ser	Phe	Gly	Leu	
	205						210					215				
cac	gcc	cgc	ggt	gtg	gag	ctg	atg	cgc	ttc	acc	acg	ccc	ggc	ttc	cct	724
His	Ala	Arg	Gly	Val	Glu	Leu	Met	Arg	Phe	Thr	Thr	Pro	Gly	Phe	Pro	
	220					225					230					
gac	agc	ccc	tac	ccc	gct	cat	gcc	cgc	tgc	cag	tgg	gcc	ctg	cgg	ggg	772
Asp	Ser	Pro	Tyr	Pro	Ala	His	Ala	Arg	Cys	Gln	Trp	Ala	Leu	Arg	Gly	
235					240				245					250		
gac	gcc	gac	tca	gtg	ctg	agc	ctc	acc	ttc	cgc	agc	ttt	gac	ctt	gcg	820
Asp	Ala	Asp	Ser	Val	Leu	Ser	Leu	Thr	Phe	Arg	Ser	Phe	Asp	Leu	Ala	
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Ser	Cys	Asp	Glu	Arg	Gly	Ser	Asp	Leu	Val	Thr	Val	Tyr	Asn	Thr	Leu	
			270					275					280			
agc	ccc	atg	gag	ccc	cac	gcc	ctg	gtg	cag	ttg	tgt	ggc	acc	tac	cct	916
Ser	Pro	Met	Glu	Pro	His	Ala	Leu	Val	Gln	Leu	Cys	Gly	Thr	Tyr	Pro	
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ccc tcc tac aac ctg acc ttc cac tcc tcc cag aac gtc ctg ctc atc Pro Ser Tyr Asn Leu Thr Phe His Ser Ser Gln Asn Val Leu Leu Ile 300 305 310	964
aca ctg ata acc aac act gag cgg cgg cat ccc ggc ttt gag gcc acc Thr Leu Ile Thr Asn Thr Glu Arg Arg His Pro Gly Phe Glu Ala Thr 315 320 325 330	1012
ttc ttc cag ctg cct agg atg agc agc tgt gga ggc cgc tta cgt aaa Phe Phe Gln Leu Pro Arg Met Ser Ser Cys Gly Gly Arg Leu Arg Lys 335 340 345	1060
gcc cag ggg aca ttc aac agc ccc tac tac cca ggc cac tac cca ccc Ala Gln Gly Thr Phe Asn Ser Pro Tyr Tyr Pro Gly His Tyr Pro Pro 350 355 360	1108
aac att gac tgc aca tgg aac att gag gtg ccc aac aac cag cat gtg Asn Ile Asp Cys Thr Trp Asn Ile Glu Val Pro Asn Asn Gln His Val 365 370 375	1156
aag gtg agc ttc aaa ttc ttc tac ctg ctg gag ccc ggc gtg cct gcg Lys Val Ser Phe Lys Phe Phe Tyr Leu Leu Glu Pro Gly Val Pro Ala 380 385 390	1204
ggc acc tgc ccc aag gac tac gtg gag atc aat ggg gag aaa tac tgc Gly Thr Cys Pro Lys Asp Tyr Val Glu Ile Asn Gly Glu Lys Tyr Cys 395 400 405 410	1252
gga gag agg tcc cag ttc gtc gtc acc agc aac agc aac aag atc aca Gly Glu Arg Ser Gln Phe Val Val Thr Ser Asn Ser Asn Lys Ile Thr 415 420 425	1300
gtt cgc ttc cac tca gat cag tcc tac acc gac acc ggc ttc tta gct Val Arg Phe His Ser Asp Gln Ser Tyr Thr Asp Thr Gly Phe Leu Ala 430 435 440	1348
gaa tac ctc tcc tac gac tcc agt gac cca tgc ccg ggg cag ttc acg Glu Tyr Leu Ser Tyr Asp Ser Ser Asp Pro Cys Pro Gly Gln Phe Thr 445 450 455	1396
tgc cgc acg ggg cgg tgt atc cgg aag gag ctg cgc tgt gat ggc tgg Cys Arg Thr Gly Arg Cys Ile Arg Lys Glu Leu Arg Cys Asp Gly Trp 460 465 470	1444
gcc gac tgc acc gac cac agc gat gag ctc aac tgc agt tgc gac gcc Ala Asp Cys Thr Asp His Ser Asp Glu Leu Asn Cys Ser Cys Asp Ala 475 480 485 490	1492
ggc cac cag ttc acg tgc aag aac aag ttc tgc aag ccc ctc ttc tgg Gly His Gln Phe Thr Cys Lys Asn Lys Phe Cys Lys Pro Leu Phe Trp 495 500 505	1540
gtc tgc gac agt gtg aac gac tgc gga gac aac agc gac gag cag ggg Val Cys Asp Ser Val Asn Asp Cys Gly Asp Asn Ser Asp Glu Gln Gly 510 515 520	1588
tgc agt tgt ccg gcc cag acc ttc agg tgt tcc aat ggg aag tgc ctc Cys Ser Cys Pro Ala Gln Thr Phe Arg Cys Ser Asn Gly Lys Cys Leu 525 530 535	1636

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tcg aaa agc cag cag tgc aat ggg aag gac gac tgt ggg gac ggg tcc Ser Lys Ser Gln Gln Cys Asn Gly Lys Asp Asp Cys Gly Asp Gly Ser 540 545 550	1684
gac gag gcc tcc tgc ccc aag gtg aac gtc gtc act tgt acc aaa cac Asp Glu Ala Ser Cys Pro Lys Val Asn Val Val Thr Cys Thr Lys His 555 560 565 570	1732
acc tac cgc tgc ctc aat ggg ctc tgc ttg agc aag ggc aac cct gag Thr Tyr Arg Cys Leu Asn Gly Leu Cys Leu Ser Lys Gly Asn Pro Glu 575 580 585	1780
tgt gac ggg aag gag gac tgt agc gac ggc tca gat gag aag gac tgc Cys Asp Gly Lys Glu Asp Cys Ser Asp Gly Ser Asp Glu Lys Asp Cys 590 595 600	1828
gac tgt ggg ctg cgg tca ttc acg aga cag gct cgt gtt gtt ggg ggc Asp Cys Gly Leu Arg Ser Phe Thr Arg Gln Ala Arg Val Val Gly Gly 605 610 615	1876
acg gat gcg gat gag ggc gag tgg ccc tgg cag gta agc ctg cat gct Thr Asp Ala Asp Glu Gly Glu Trp Pro Trp Gln Val Ser Leu His Ala 620 625 630	1924
ctg ggc cag ggc cac atc tgc ggt gct tcc ctc atc tct ccc aac tgg Leu Gly Gln Gly His Ile Cys Gly Ala Ser Leu Ile Ser Pro Asn Trp 635 640 645 650	1972
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tca gac ccc acg cag tgg acg gcc ttc ctg ggc ttg cac gac cag agc Ser Asp Pro Thr Gln Trp Thr Ala Phe Leu Gly Leu His Asp Gln Ser 670 675 680	2068
cag cgc agc gcc cct ggg gtg cag gag cgc agg ctc aag cgc atc atc Gln Arg Ser Ala Pro Gly Val Gln Glu Arg Arg Leu Lys Arg Ile Ile 685 690 695	2116
tcc cac ccc ttc ttc aat gac ttc acc ttc gac tat gac atc gcg ctg Ser His Pro Phe Phe Asn Asp Phe Thr Phe Asp Tyr Asp Ile Ala Leu 700 705 710	2164
ctg gag ctg gag aaa ccg gca gag tac agc tcc atg gtg cgg ccc atc Leu Glu Leu Glu Lys Pro Ala Glu Tyr Ser Ser Met Val Arg Pro Ile 715 720 725 730	2212
tgc ctg ccg gac gcc tcc cat gtc ttc cct gcc ggc aag gcc atc tgg Cys Leu Pro Asp Ala Ser His Val Phe Pro Ala Gly Lys Ala Ile Trp 735 740 745	2260
gtc acg ggc tgg gga cac acc cag tat gga ggc act ggc gcg ctg atc Val Thr Gly Trp Gly His Thr Gln Tyr Gly Gly Thr Gly Ala Leu Ile 750 755 760	2308
ctg caa aag ggt gag atc cgc gtc atc aac cag acc acc tgc gag aac Leu Gln Lys Gly Glu Ile Arg Val Ile Asn Gln Thr Thr Cys Glu Asn	2356

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765	770	775	
ctc ctg ccg cag cag atc acg ccg cgc atg atg tgc gtg ggc ttc ctc			2404
Leu Leu Pro Gln Gln Ile Thr Pro Arg Met Met Cys Val Gly Phe Leu			
780	785	790	
agc ggc ggc gtg gac tcc tgc cag ggt gat tcc ggg gga ccc ctg tcc			2452
Ser Gly Gly Val Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Ser			
795	800	805	810
agc gtg gag gcg gat ggg cgg atc ttc cag gcc ggt gtg gtg agc tgg			2500
Ser Val Glu Ala Asp Gly Arg Ile Phe Gln Ala Gly Val Val Ser Trp			
	815	820	825
gga gac ggc tgc gct cag agg aac aag cca ggc gtg tac aca agg ctc			2548
Gly Asp Gly Cys Ala Gln Arg Asn Lys Pro Gly Val Tyr Thr Arg Leu			
	830	835	840
cct ctg ttt cgg gac tgg atc aaa gag aac act ggg gta ta ggggccgggg			2599
Pro Leu Phe Arg Asp Trp Ile Lys Glu Asn Thr Gly Val			
	845	850	855
ccacccaaat gtgtacacct gcggggccac ccacgtccca cccagtggtg cagcctgca			2659
ggctggagac tggaccgctg actgcaccag cgccccaga acatacactg tgaactcaat			2719
ctccagggtc ccaaactctgc ctagaaaacc tctcgttcc tcagcctcca aagtggagct			2779
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aaaaaaaa			3147
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<213> Homo Sapien			
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20 25 30			
Glu Glu Gly Val Glu Phe Leu Pro Val Asn Asn Val Lys Lys Val Glu			
35 40 45			
Lys His Gly Pro Gly Arg Trp Val Val Leu Ala Ala Val Leu Ile Gly			
50 55 60			
Leu Leu Leu Val Leu Leu Gly Ile Gly Phe Leu Val Trp His Leu Gln			
65 70 75 80			
Tyr Arg Asp Val Arg Val Gln Lys Val Phe Asn Gly Tyr Met Arg Ile			
85 90 95			
Thr Asn Glu Asn Phe Val Asp Ala Tyr Glu Asn Ser Asn Ser Thr Glu			
100 105 110			
Phe Val Ser Leu Ala Ser Lys Val Lys Asp Ala Leu Lys Leu Leu Tyr			
115 120 125			
Ser Gly Val Pro Phe Leu Gly Pro Tyr His Lys Glu Ser Ala Val Thr			
130 135 140			
Ala Phe Ser Glu Gly Ser Val Ile Ala Tyr Tyr Trp Ser Glu Phe Ser			
145 150 155 160			

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Ile Pro Gln His Leu Val Glu Glu Ala Glu Arg Val Met Ala Glu Glu
 165 170 175
 Arg Val Val Met Leu Pro Pro Arg Ala Arg Ser Leu Lys Ser Phe Val
 180 185 190
 Val Thr Ser Val Val Ala Phe Pro Thr Asp Ser Lys Thr Val Gln Arg
 195 200 205
 Thr Gln Asp Asn Ser Cys Ser Phe Gly Leu His Ala Arg Gly Val Glu
 210 215 220
 Leu Met Arg Phe Thr Thr Pro Gly Phe Pro Asp Ser Pro Tyr Pro Ala
 225 230 235 240
 His Ala Arg Cys Gln Trp Ala Leu Arg Gly Asp Ala Asp Ser Val Leu
 245 250 255
 Ser Leu Thr Phe Arg Ser Phe Asp Leu Ala Ser Cys Asp Glu Arg Gly
 260 265 270
 Ser Asp Leu Val Thr Val Tyr Asn Thr Leu Ser Pro Met Glu Pro His
 275 280 285
 Ala Leu Val Gln Leu Cys Gly Thr Tyr Pro Pro Ser Tyr Asn Leu Thr
 290 295 300
 Phe His Ser Ser Gln Asn Val Leu Leu Ile Thr Leu Ile Thr Asn Thr
 305 310 315 320
 Glu Arg Arg His Pro Gly Phe Glu Ala Thr Phe Phe Gln Leu Pro Arg
 325 330 335
 Met Ser Ser Cys Gly Gly Arg Leu Arg Lys Ala Gln Gly Thr Phe Asn
 340 345 350
 Ser Pro Tyr Tyr Pro Gly His Tyr Pro Pro Asn Ile Asp Cys Thr Trp
 355 360 365
 Asn Ile Glu Val Pro Asn Asn Gln His Val Lys Val Ser Phe Lys Phe
 370 375 380
 Phe Tyr Leu Leu Glu Pro Gly Val Pro Ala Gly Thr Cys Pro Lys Asp
 385 390 395 400
 Tyr Val Glu Ile Asn Gly Glu Lys Tyr Cys Gly Glu Arg Ser Gln Phe
 405 410 415
 Val Val Thr Ser Asn Ser Asn Lys Ile Thr Val Arg Phe His Ser Asp
 420 425 430
 Gln Ser Tyr Thr Asp Thr Gly Phe Leu Ala Glu Tyr Leu Ser Tyr Asp
 435 440 445
 Ser Ser Asp Pro Cys Pro Gly Gln Phe Thr Cys Arg Thr Gly Arg Cys
 450 455 460
 Ile Arg Lys Glu Leu Arg Cys Asp Gly Trp Ala Asp Cys Thr Asp His
 465 470 475 480
 Ser Asp Glu Leu Asn Cys Ser Cys Asp Ala Gly His Gln Phe Thr Cys
 485 490 495
 Lys Asn Lys Phe Cys Lys Pro Leu Phe Trp Val Cys Asp Ser Val Asn
 500 505 510
 Asp Cys Gly Asp Asn Ser Asp Glu Gln Gly Cys Ser Cys Pro Ala Gln
 515 520 525
 Thr Phe Arg Cys Ser Asn Gly Lys Cys Leu Ser Lys Ser Gln Gln Cys
 530 535 540
 Asn Gly Lys Asp Asp Cys Gly Asp Gly Ser Asp Glu Ala Ser Cys Pro
 545 550 555 560
 Lys Val Asn Val Val Thr Cys Thr Lys His Thr Tyr Arg Cys Leu Asn
 565 570 575
 Gly Leu Cys Leu Ser Lys Gly Asn Pro Glu Cys Asp Gly Lys Glu Asp
 580 585 590
 Cys Ser Asp Gly Ser Asp Glu Lys Asp Cys Asp Cys Gly Leu Arg Ser
 595 600 605
 Phe Thr Arg Gln Ala Arg Val Val Gly Gly Thr Asp Ala Asp Glu Gly
 610 615 620
 Glu Trp Pro Trp Gln Val Ser Leu His Ala Leu Gly Gln Gly His Ile

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<212> DNA
<213> Homo Sapien
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<222> (1865)...(2590)
<223> Nucleic acid sequence of protease domain of MTSP1
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[illegible]

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caacaaccag	catgtgaagg	tgagcttcaa	attcttctac	ctgctggagc	ccggcgctgcc	1200
tgccgggcacc	tgccccaagg	actacgtgga	gatcaatggg	gagaaatact	gcggagagag	1260
gtcccagttc	gtcgtcacca	gcaacagcaa	caagatcaca	gttcgcttcc	actcagatca	1320
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cccggggcag	ttcacgtgcc	gcacggggcg	gtgtatccgg	aaggagctgc	gctgtgatgg	1440
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tagcgacggc	tcagatgaga	aggactgcga	ctgtgggctg	cggtcattca	cgagacaggc	1860
tcgt gtt gtt	ggg acg gat	gcg gat	gag ggc	gag tgg	ccc tgg	cag 1909
Val Val Gly Gly	Thr Asp Ala	Asp Glu Gly	Glu Trp Pro	Trp Gln		
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gta agc ctg cat gct ctg ggc cag ggc cac atc tgc ggt gct tcc ctg	1957					
Val Ser Leu His Ala Leu Gly Gln Gly His Ile Cys Gly Ala Ser Leu						
20	25	30				
atc tct ccc aac tgg ctg gtc tct gcc gca cac tgc tac atc gat gac	2005					
Ile Ser Pro Asn Trp Leu Val Ser Ala Ala His Cys Tyr Ile Asp Asp						
35	40	45				
aga gga ttc agg tac tca gac ccc acg cag tgg acg gcc ttc ctg ggc	2053					
Arg Gly Phe Arg Tyr Ser Asp Pro Thr Gln Trp Thr Ala Phe Leu Gly						
50	55	60				
ttg cac gac cag agc cag cgc agc gcc cct ggg gtg cag gag cgc agg	2101					
Leu His Asp Gln Ser Gln Arg Ser Ala Pro Gly Val Gln Glu Arg Arg						
65	70	75				
ctc aag cgc atc atc tcc cac ccc ttc ttc aat gac ttc acc ttc gac	2149					
Leu Lys Arg Ile Ile Ser His Pro Phe Phe Asn Asp Phe Thr Phe Asp						
80	85	90	95			
tat gac atc gcg ctg ctg gag ctg gag aaa ccg gca gag tac agc tcc	2197					
Tyr Asp Ile Ala Leu Leu Glu Leu Glu Lys Pro Ala Glu Tyr Ser Ser						
100	105	110				
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Met Val Arg Pro Ile Cys Leu Pro Asp Ala Ser His Val Phe Pro Ala						
115	120	125				
ggc aag gcc atc tgg gtc acg ggc tgg gga cac acc cag tat gga ggc	2293					
Gly Lys Ala Ile Trp Val Thr Gly Trp Gly His Thr Gln Tyr Gly Gly						
130	135	140				
act ggc gcg ctg atc ctg caa aag ggt gag atc cgc gtc atc aac cag	2341					
Thr Gly Ala Leu Ile Leu Gln Lys Gly Glu Ile Arg Val Ile Asn Gln						
145	150	155				
acc acc tgc gag aac ctc ctg ccg cag cag atc acg ccg cgc atg atg	2389					
Thr Thr Cys Glu Asn Leu Leu Pro Gln Gln Ile Thr Pro Arg Met Met						
160	165	170	175			
tgc gtg ggc ttc ctc agc ggc ggc gtg gac tcc tgc cag ggt gat tcc	2437					
Cys Val Gly Phe Leu Ser Gly Gly Val Asp Ser Cys Gln Gly Asp Ser						

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180	185	190	
ggg gga ccc ctg tcc agc gtg gag gcg gat ggg cgg atc ttc cag gcc			2485
Gly Gly Pro Leu Ser Ser Val Glu Ala Asp Gly Arg Ile Phe Gln Ala			
195	200	205	
ggt gtg gtg agc tgg gga gac ggc tgc gct cag agg aac aag cca ggc			2533
Gly Val Val Ser Trp Gly Asp Gly Cys Ala Gln Arg Asn Lys Pro Gly			
210	215	220	
gtg tac aca agg ctc cct ctg ttt cgg gac tgg atc aaa gag aac act			2581
Val Tyr Thr Arg Leu Pro Leu Phe Arg Asp Trp Ile Lys Glu Asn Thr			
225	230	235	
ggg gta tag gggccggggc caccctaatg tgtacacctg cggggccacc			2630
Gly Val *			
240			
catcgctccac cccagtgtgc acgcctgcag gctggagact ggaccgctga ctgcaccagc			2690
gccccccagaa catacactgt gaactcaatc tccagggctc caaatctgcc tagaaaacct			2750
ctcgcttctct cagcctccaa agtggagctg ggaggttagaa ggggaggaca ctggtggttc			2810
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Ser Pro Asn Trp Leu Val Ser Ala Ala His Cys Tyr Ile Asp Asp Arg			
35 40 45			
Gly Phe Arg Tyr Ser Asp Pro Thr Gln Trp Thr Ala Phe Leu Gly Leu			
50 55 60			
His Asp Gln Ser Gln Arg Ser Ala Pro Gly Val Gln Glu Arg Arg Leu			
65 70 75 80			
Lys Arg Ile Ile Ser His Pro Phe Phe Asn Asp Phe Thr Phe Asp Tyr			
85 90 95			
Asp Ile Ala Leu Leu Glu Leu Glu Lys Pro Ala Glu Tyr Ser Ser Met			
100 105 110			
Val Arg Pro Ile Cys Leu Pro Asp Ala Ser His Val Phe Pro Ala Gly			
115 120 125			
Lys Ala Ile Trp Val Thr Gly Trp Gly His Thr Gln Tyr Gly Gly Thr			
130 135 140			
Gly Ala Leu Ile Leu Gln Lys Gly Glu Ile Arg Val Ile Asn Gln Thr			
145 150 155 160			
Thr Cys Glu Asn Leu Leu Pro Gln Gln Ile Thr Pro Arg Met Met Cys			
165 170 175			
Val Gly Phe Leu Ser Gly Gly Val Asp Ser Cys Gln Gly Asp Ser Gly			
180 185 190			
Gly Pro Leu Ser Ser Val Glu Ala Asp Gly Arg Ile Phe Gln Ala Gly			

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      195      200      205
Val Val Ser Trp Gly Asp Gly Cys Ala Gln Arg Asn Lys Pro Gly Val
      210      215      220
Tyr Thr Arg Leu Pro Leu Phe Arg Asp Trp Ile Lys Glu Asn Thr Gly
225      230      235      240
Val

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<210> 5
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<212> DNA
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<220>
<221> CDS
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<223> Nucleotide sequence encoding CVSP14 protease
      domain

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tct ctg aaa caa agg cag aag cat att tgt gga gga agc atc gtc tca      96
Ser Leu Lys Gln Arg Gln Lys His Ile Cys Gly Gly Ser Ile Val Ser
      20          25          30

cca cag tgg gtg atc acg gcg gct cac tgc att gca aac aga aac att      144
Pro Gln Trp Val Ile Thr Ala Ala His Cys Ile Ala Asn Arg Asn Ile
      35          40          45

gtg tct act ttg aat gtt act gct gga gag tat gac tta agc cag aca      192
Val Ser Thr Leu Asn Val Thr Ala Gly Glu Tyr Asp Leu Ser Gln Thr
      50          55          60

gac cca gga gag caa act ctc act att gaa act gtc atc ata cat cca      240
Asp Pro Gly Glu Gln Thr Leu Thr Ile Glu Thr Val Ile Ile His Pro
      65          70          75          80

cat ttc tcc acc aag aaa cca atg gac tat gat att gcc ctt ttg aag      288
His Phe Ser Thr Lys Lys Pro Met Asp Tyr Asp Ile Ala Leu Leu Lys
      85          90          95

atg get gga gcc ttc caa ttt ggc cac ttt gtg ggg ccc ata tgt ctt      336
Met Ala Gly Ala Phe Gln Phe Gly His Phe Val Gly Pro Ile Cys Leu
      100          105          110

cca gag ctg cgg gag caa ttt gag gct ggt ttt att tgt aca act gca      384
Pro Glu Leu Arg Glu Gln Phe Glu Ala Gly Phe Ile Cys Thr Thr Ala
      115          120          125

ggc tgg ggc cgc tta act gaa ggt ggc gtc ctc tca caa gtc ttg cag      432
Gly Trp Gly Arg Leu Thr Gln Gly Gly Val Leu Ser Gln Val Leu Gln
      130          135          140

gaa gtg aat ctg cct att ttg acc tgg gaa gag tgt gtg gca gct ctg      480
Glu Val Asn Leu Pro Ile Leu Thr Trp Glu Glu Cys Val Ala Ala Leu
145          150          155          160

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tta aca cta aag agg ccc atc agt ggg aag acc ttt ctt tgc aca ggt      528
Leu Thr Leu Lys Arg Pro Ile Ser Gly Lys Thr Phe Leu Cys Thr Gly
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Pro Gln Trp Val Ile Thr Ala Ala His Cys Ile Ala Asn Arg Asn Ile
35      40      45
Val Ser Thr Leu Asn Val Thr Ala Gly Glu Tyr Asp Leu Ser Gln Thr
50      55      60
Asp Pro Gly Glu Gln Thr Leu Thr Ile Glu Thr Val Ile Ile His Pro
65      70      75      80
His Phe Ser Thr Lys Lys Pro Met Asp Tyr Asp Ile Ala Leu Leu Lys
85      90      95
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Pro Glu Leu Arg Glu Gln Phe Glu Ala Gly Phe Ile Cys Thr Thr Ala
115     120     125
Gly Trp Gly Arg Leu Thr Glu Gly Gly Val Leu Ser Gln Val Leu Gln
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Glu Val Asn Leu Pro Ile Leu Thr Trp Glu Glu Cys Val Ala Ala Leu
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Leu Thr Leu Lys Arg Pro Ile Ser Gly Lys Thr Phe Leu Cys Thr Gly
165     170     175
Phe Pro Asp Gly Gly Arg Asp Ala Cys Gln Gly Asp Ser Gly Gly Ser
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<221> modified base
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28

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29

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<211> 9276
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<211> 1035

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<221> CDS

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<223> DNA encoding full length CVSP14

<221> misc_signal

<222> (115)...(189)

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gtg ggg ccc ata tgt ctt cca gag ctg cgg gag caa ttt gag gct ggt	645
Val Gly Pro Ile Cys Leu Pro Glu Leu Arg Glu Gln Phe Glu Ala Gly	
165 170 175	
ttt att tgt aca act gca ggc tgg ggc cgc tta act gaa ggt ggc gtc	693
Phe Ile Cys Thr Thr Ala Gly Trp Gly Arg Leu Thr Glu Gly Gly Val	
180 185 190	
ctc tca caa gtc ttg cag gaa gtg aat ctg cct att ttg acc tgg gaa	741
Leu Ser Gln Val Leu Gln Val Asn Leu Pro Ile Leu Thr Trp Glu	
195 200 205	
gag tgt gtg gca gct ctg tta aca cta aag agg ccc atc agt ggg aag	789
Glu Cys Val Ala Ala Leu Thr Leu Lys Arg Pro Ile Ser Gly Lys	
210 215 220 225	

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acc ttt ctt tgc aca ggt ttt cct gat gga ggg aga gac gca tgt cag      837
Thr Phe Leu Cys Thr Gly Phe Pro Asp Gly Gly Arg Asp Ala Cys Gln
                230                235                240

gga gat tca gga ggt tca ctc atg tgc cgg aat aag aaa ggg gcc tgg      885
Gly Asp Ser Gly Gly Ser Leu Met Cys Arg Asn Lys Lys Gly Ala Trp
                245                250                255

act ctg gct ggt gtg act tcc tgg ggt ttg ggc tgt ggt cga ggc tgg      933
Thr Leu Ala Gly Val Thr Ser Trp Gly Leu Gly Cys Gly Arg Gly Trp
                260                265                270

aga aac aat gtg agg aaa agt gat caa gga tcc cct ggg atc ttc aca      981
Arg Asn Asn Val Arg Lys Ser Asp Gln Gly Ser Pro Gly Ile Phe Thr
                275                280                285

gac att agt aaa gtg ctt tcc tgg atc cac gaa cac atc caa act ggt      1029
Asp Ile Ser Lys Val Leu Ser Trp Ile His Glu His Ile Gln Thr Gly
290                295                300                305

aac taa
Asn *

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1035

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 20      25      30
Lys Ala Pro Ser Cys Gly Gln Ser Leu Val Lys Val Gln Pro Trp Asn
 35      40      45
Tyr Phe Asn Ile Phe Ser Arg Ile Leu Gly Gly Ser Gln Val Glu Lys
 50      55      60
Gly Ser Tyr Pro Trp Gln Val Ser Leu Lys Gln Arg Gln Lys His Ile
 65      70      75      80
Cys Gly Gly Ser Ile Val Ser Pro Gln Trp Val Ile Thr Ala Ala His
 85      90      95
Cys Ile Ala Asn Arg Asn Ile Val Ser Thr Leu Asn Val Thr Ala Gly
100      105      110
Glu Tyr Asp Leu Ser Gln Thr Asp Pro Gly Glu Gln Thr Leu Thr Ile
115      120      125
Glu Thr Val Ile Ile His Pro His Phe Ser Thr Lys Lys Pro Met Asp
130      135      140
Tyr Asp Ile Ala Leu Leu Lys Met Ala Gly Ala Phe Gln Phe Gly His
145      150      155      160
Phe Val Gly Pro Ile Cys Leu Pro Glu Leu Arg Glu Gln Phe Glu Ala
165      170      175
Gly Phe Ile Cys Thr Thr Ala Gly Trp Gly Arg Leu Thr Glu Gly Gly

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[illegible]

39

33

39

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<223> primer